ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 23 DECEMBER, 1938 NUMBER 6

DIABETES MELLITUS ASSOCIATED WITH HYPERTHYROIDISM[1]

DANIEL P. FOSTER AND WILLIAM L. LOWRIE, JR.
From the Metabolic Division of the Department of Medicine of the Henry Ford Hospital
DETROIT, MICHIGAN

A well-established clinical rule has been stated many times that, whenever possible, the clinical signs and symptoms of a patient should be explained with a single diagnosis. This rule breaks down most frequently when dealing with the insidious symptoms of chronic disease in contrast with the dramatic manifestations of acute illness.

The differential diagnosis of diabetes mellitus and hyperthyroidism is particularly meticulous. This fact is explained by the similarity or actual identity of cardinal symptoms of the diseases. Polyphagia, polydypsia, weight loss, and strength loss are common factors in both diabetes mellitus and hyperthyroidism. Glucose is frequently found in the urine of the patient with hyperthroidism, and hyperglycemia is common. The glycosuria and hyperglycemia are due, however, to a different mechanism in each of the two diseases. In hyperthyroidism, glycogenolysis is accelerated and, in diabetes, oxidation of glucose is insufficient. In both instances, the liver is rendered low in glycogen. Adequate treatment tends to correct this.

Most authors on this subject (1, 2, 3, 4, 5) are agreed that the fasting blood-sugar levels acceptable for a diagnosis of diabetes in the presence of hyperthyroidism should be increased to 160 mg. per 100 cc. of blood or more, and that blood sugars after food should be at least 200 mg. per 100 cc. of blood. We agree to these values, and also with the impression that a normal tolerance for carbohydrate after thyroidectomy probably indicates an erroneous diagnosis of diabetes mellitus. Wilder (6) states that the severity of a diabetes is lessened after total ablation of the thyroid, but no instance of the disappearance of the disease has been recorded. Furthermore, it is much safer to consider high blood sugars diagnostic only when the previous carbohydrate intake is known. Much is to be said for a test meal and a blood-sugar estimation 2½ hours later.

[1] Read before the 21st Annual Meeting of The Association for the Study of Internal Secretions, San Francisco, Calif.

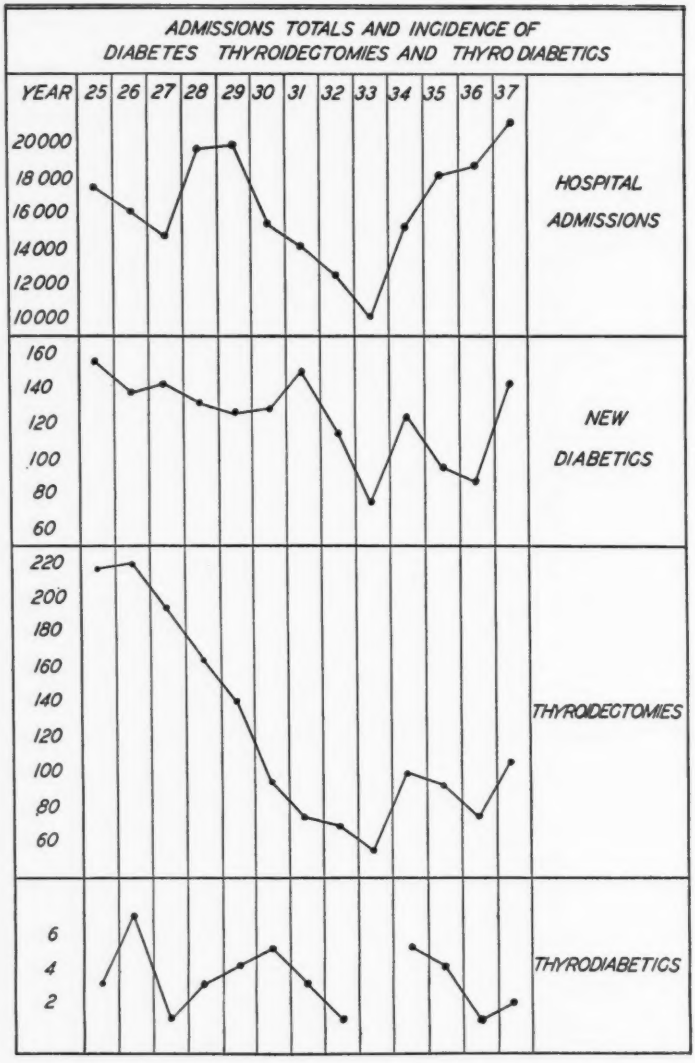


Fig. 1. Admissions totals and incidence of diabetes, thyroidectomies and thyrodiabetics.

When the two diseases co-exist, the diagnosis of diabetes mellitus is usually made first. Certain features during the course of the illness arouse suspicion that thyroid disease may be a complicating factor. An inability to control the blood sugar and glycosuria, with what seems to be a suitable diet and insulin dosage, or the failure of a patient to hold his weight, or to gain while consuming a diet adequate for either circumstance is significant. These patients also are prone to develop acidosis, especially if they are losing weight or are undernourished. A rapid pulse, intolerance to heat or bed-clothes, restless-

ness, flushing, red elbows if the patient is in bed, apprehension expressed and observed, a moist skin, and eye signs are all aids to a correct diagnosis, particularly in the younger patient. The older patient, who most frequently does not display the common clinical signs of hyperthyroidism, is more difficult to evaluate, and is most frequently discovered because his diabetes is hard to control. Acute or chronic infection, of course, is the most common reason for this circumstance, and is carefully excluded.

We have a feeling that the division of these cases into the groupings of primary hyperthyroidism and secondary hyperthyroidism, or patients with hyperplastic thyroids and adenomatous goiters is, from a metabolic consideration, not significant. The B.M.R. of the cases of adenomatous goiter is, as a rule, less elevated than is observed in patients with hyperplastic goiter. The age incidence of these two types of thyroid disease would influence the frequency of their association with diabetes mellitus. Diabetes occurs more frequently in the decades which correspond to the onset of the symptoms due to toxic adenomata. Wilder did note, however, that patients with toxic adenomata showed a higher incidence of hyperglycemia (20 per cent) than those with hyperplasia of the gland. Postoperative improvement is similar after thyroidectomy in both groups.

Material Studied

The group of thyrodiabetics here studied comprises 42 cases. There was a total of 45 operations. Thirty-six cases are analyzed completely in the main study. The remaining 6 are not included in the main study because they were operated upon elsewhere, or a long period had elapsed since operation. All members of this latter group had blood-sugar elevations or decreased glucose-tolerance tests. They had had a primary diagnosis of hyperthyroidism, and the diabetes was suspected because of glycosuria or pruritis vulvae, or some other secondary symptom of diabetes. Of the 6, one was a male and 5 were females. The type of goiter could not be determined. All had diabetes after operation and 4 required insulin. At time of operation, one patient was between 31 and 40 years, two between 41 and 50 years, two between 51 and 60 years, and one between 61 and 70 years of age. Their pulse rates, state of nutrition, and relative carbohydrate tolerance could not be estimated. One patient had a

TABLE 1. THYROIDECTOMIES, DIABETICS, THYRODIABETICS IN RELATION TO ADMISSIONS

	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
Admissions	17336	16110	14958	19680	19927	15586	14127	12774	10108	15202	18375	18878	21162
Diabetes	155	139	143	132	126	129	150	115	74	125	96	88	144
Thyroidectomies	218	220	196	156	141	97	76	70	57	100	94	75	107
Thyroidectomies in diabetics	3	7	1	3	4	5	3	1	0	5	4	1	2

13-YEAR TOTAL

Admissions	214,283	
Diabetes	1,616	2.41%
Thyroidectomies	1,607	2.43%
Thyrodiabetics	42[1]	

[1] Includes two thyroidectomies in 1924, one in 1919.

B.M.R. of —1, another between +1 and +10, two between +11 and +20, and one between +21 and +30.

The validity of the diagnosis of hyperthyroidism might be challenged on reviewing the cases having metabolic rates below +10. In Michigan, many patients receive iodine in some form or another before reaching the hospital, and the B.M.R. observed is at times depressed below the true reading without such treatment. Diagnosis is coming to be predominately more clinical than laboratory.

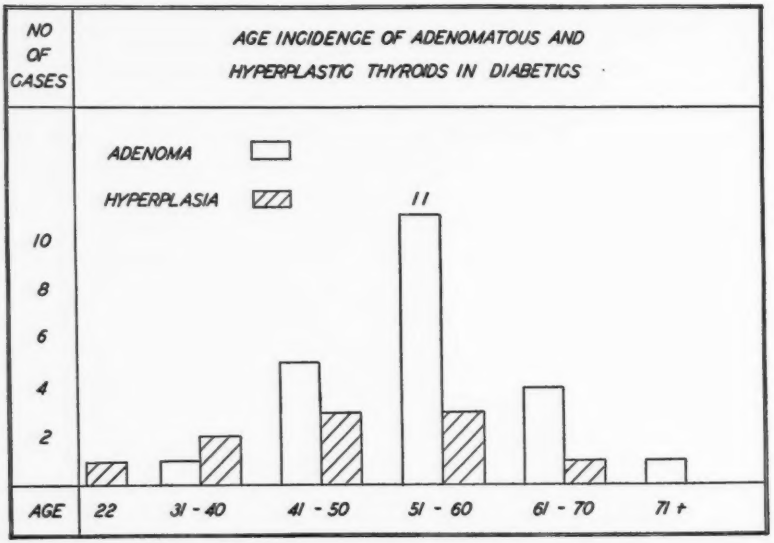


Fig. 2. Age incidence of adenomatous and hyperplastic thyroids in diabetics.

Statistical analysis of incidence. These cases of diabetes associated with hyperthyroidism were gleaned from the admissions of the Henry Ford Hospital from 1925 to 1938. During that period, there were 214,223 new cases examined. Of these, 1616 were diabetic, and a total of 1607 operations for hyperthyroidism were done. There were discovered 42 patients with hyperthyroidism associated with diabetes mellitus.

A year-to-year analysis of these admissions is very interesting. The curve of admissions varies with the curves of financial depression and recoveries

TABLE 2. AGE INCIDENCE OF HYPERTHYROIDISM IN DIABETICS

	No.
21 to 30 yr.	1
31 to 40 yr.	3
41 to 50 yr.	7
51 to 60 yr.	13
61 to 70 yr.	10
71 and over	2
Total	36

and recessions. The curve of diabetic admissions roughly parallels the admission curve.

Of especial interest is the thyroidectomy curve, for there has been, since 1929, a marked drop in the frequency of thyroidectomy. This drop has been discussed by McClure (8) and has been, we believe, rightly attributed to the introduction, in 1926, of iodine into the table salt sold in Michigan. The number of thyrodiabetics is too small to make profitable a year-to-year analysis of its incidence.

Of diabetic admissions, 2.41 per cent had this disease complicated with hyperthyroidism of all types. Of the patients with a primary diagnosis of hyperthyroidism, 2.43 per cent had this disease in combination with diabetes mellitus.

The age distribution of the 36 cases is shown in table 2. The largest percentage of the group was from 51 to 70 years of age, only 12 cases falling

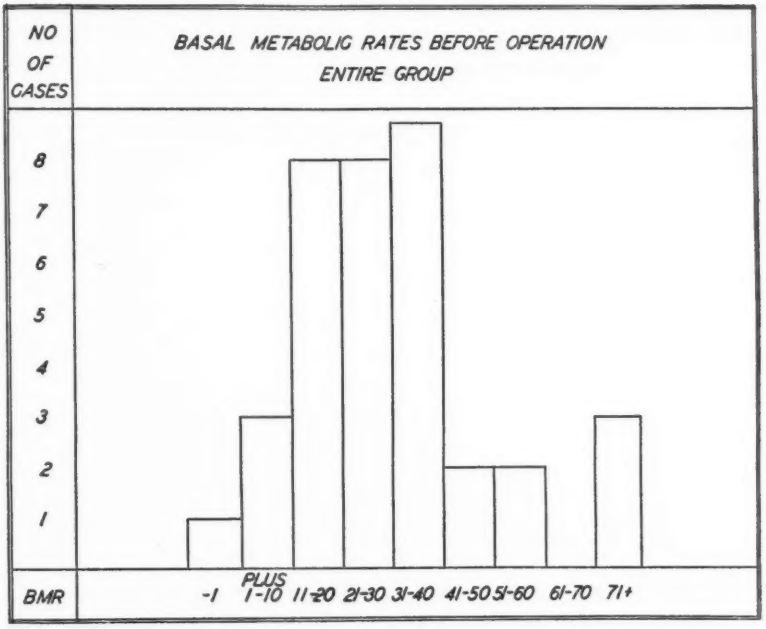


Fig. 3. B.M.R. before operation; entire group.

outside these limits. Both diabetes and adenomatous goiter are more prevalent in the sixth and seventh decades, so this distribution is not surprising.

The sex distribution was significantly predominately female (28 cases, 80 per cent); 20 per cent (8 cases) occurred in males. A family history of diabetes was elicited in only 9 patients (25 per cent).

Twenty-five patients were born or lived in a goiter region before puberty, and 7 were born or raised in a non-goiter region. The birthplaces of 10 were not recorded. These facts are of particular significance when comparing the

TABLE 3. ANALYSIS OF DEATHS

No.	Age	Survival Period	Cause of Death	Autopsy
1	67	2 days	Auricular thrombi pneumonia	Yes
2	56	3 mo.	Coronary occlusion	0
3	66	2 yr.	Heat exhaustion	Yes
4	66	2 yr., 8 mo.	Ischiorectal abscess gangrene	0
5	69	3 yr., 6 mo.	Myocardial failure	0
6	54	5 yr., 8 mo.	Cerebral hemorrhage[1]	Yes
7	61	6 yr., 4 mo.	Acute appendicitis coronary occlusion	0
8	76	11 yr.	Arteriosclerosis myocardial degeneration	Yes Hypertrophy of adrenal cortex
9	22	4 mo.	Found dead in bed	Yes Myocarditis

[1] B.P. 248/120 before operation.

incidence of thyroid disease alone or in combination with any other disease in non-goiterous areas. In a goiterous district, one has reason to expect a higher incidence of association. This does seem to be the case. In Joslin's (7) cases 1.52 per cent of diabetics had their illness complicated with hyperthyroidism whereas in this series 2.41 per cent of diabetics were so complicated. The general average incidence in the reports in the literature to 1935 summarized by Joslin is 1.68 per cent.

In patients, reported by Joslin, who had a primary diagnosis of hyperthyroidism, 3.15 per cent had the additional diagnosis of diabetes mellitus. In this series only 2.43 per cent were so complicated. The general average summarized by Joslin was 2.31 per cent.

Duration of life. Nine cases in this series have died. One case survived ligation only 2 days. This patient was 67 years old, had a very high B.M.R. and at autopsy showed auricular thrombi and pneumonia. One case died under circumstances suggesting a suicidal or cardiac death. Omitting the pneumonia death, the average survival of those patients who have died is approximately 4 years and 1 month.

The causes of death are recorded in table 3. Five patients were autopsied and the diagnoses were confirmed. It will be noted that the cause of death is predominately vascular or that vascular disease was a complicating factor. The patient dying of heat exhaustion had marked arteriosclerosis.

TABLE 4. EXOPHTHALMOS IN RELATION TO HISTOPATHOLOGY OF GLAND

	Total Cases	With Exophthalmos
Hyperplasia	8	3
Colloid goiter	3	1
Adenoma—22 cases		
Fetal adenomata	8	
Degenerating fetal adenomata	7	3
Colloid goiter with fetal adenomata	7	1
Colloid goiter with degenerating fetal adenomata	1	
Hyperplasia + adenoma	2	1
Total	36	9

The finding of hypertrophy of the adrenal cortex in one patient is of particular significance. This patient displayed a diabetes of unusual severity and was difficult to manage. X-ray exposures as initial hyperthyroid therapy were not successful. No case died in acidosis or coma. The deaths can be listed as cardiovascular (primarily) in 4, and as a contributary factor in all 8 cases; gangrene associated with an ischiorectal abscess, 1 acute infection, 3 additional; heat exhaustion, 1; suicide or cardiac, 1 case.

Histopathology of the gland. The large majority of the cases in this series had various types of adenoma of the thyroid. Twenty-three cases, or 63.9 per cent, were so diagnosed by Dr. Frank W. Hartman. There were 3 glands classified as colloid goiter and one such case had exophthalmos. The B.M.R. of these 3 cases were +18 per cent, +24 per cent, and +19 per cent. We have come to the habit of expecting to find in patients with exophthalmos a hyperplastic type of thyroid enlargement, but 50 per cent of the cases of this series exhibiting exophthalmos had no demonstrable hyperplasia when the gland was sectioned.

TABLE 5. ELECTROCARDIOGRAPHIC FINDINGS

Total examinations	36
Normal	18
Auricular extrasystoles	1
Flat T waves	4
Inverted T waves	4
Diphasic T waves	4
Large P waves	1
Auricular fibrillation	4
Incomplete bundle branch block	1
Toxic myocarditis	1

Age incidence of adenomatous and hyperplastic thyroids in diabetics. An analysis of the age incidence of adenomatous and hyperplastic thyroids in diabetics bears out the impression that adenoma occurs more frequently in the latter decades. Only one gland could be so classified below the age of 40, and a marked predominance of adenoma occurred after the age of 50. Figure 2 illustrates this fact graphically.

Basal metabolic rates. The large majority of basal rates taken before operation fell between +11 and +40 per cent. This fact is due to the predominance of toxic adenomata in this group. Hyperplasia usually causes higher rates than these. The highest rate was observed in a patient who had acute thyroiditis and developed an acidosis while in the hospital under what would ordinarily have been adequate dietary control and insulin dosage. After the acidosis was corrected, thyroidectomy was done and the patient was discharged under control, which was possible with a maintenance diet and but 20 U of insulin.

As has been mentioned elsewhere, the low rates before operation were in most instances the result of iodine medication.

Electrocardiographic findings. Thirty-six electrocardiograms were made, and 18 could be classed as normal. This fact has interest when one considers the average age of the group, and that the patients were suffering from two diseases, both of which are to be listed as causing cardiovascular disease. The type of abnormality found, and its frequency, is shown in table 5.

Blood pressure by age groups. A study of the blood-pressure records re-

veals that, past the age of 40, hypertension is present. The type of elevation is typical of hyperthyroidism and of atherosclerosis. The diastolic pressure is not disturbed. The average pulse pressure is definitely widened, as is so characteristic in patients with over-active thyroids. Both hyperthyroidism and diabetes are recognized etiological factors for vascular damage.

Changes in carbohydrate tolerance. Changes in carbohydrate tolerance are most difficult to evaluate. The criteria for such evaluation are not clear. The level of the blood sugar and amount of urinary sugar will decrease spon-

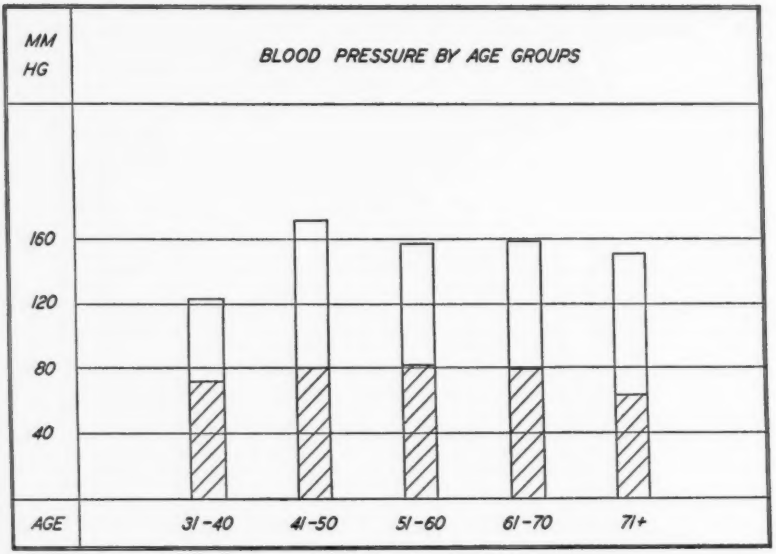


Fig. 4. B.P. by age group.

taneously with control of the diabetes, and the observed improvement may erroneously be credited to whatever procedure was carried out in the interim. Certainly, comparative diet and insulin dosage can be misleading. Blood-sugar estimations and urinary-sugar amounts do not tell the story. Patients change in weight, too, and may be clinically much improved without the usual criteria for tolerance estimation being altered. One has to be content with averages. We are certain that these patients were in better health and vigor, were better nourished, less liable to acidosis, and their diabetes more easily stabilized after operation.

Table 6 gives the average fasting blood-sugar readings before operation as 164.3 mg. per 100 cc. of blood, and after operation as 135.1 mg. per 100 cc. of blood. The insulin requirements before and after operation indicate improved carbohydrate tolerance. The diets before and after operation were very similar. The average weight gain was 16 lb. Twenty-five patients required insulin before operation, and but 19 during the hospital stay after operation. Eventually, 8 patients were able to discontinue their insulin entirely. Thirteen others required less insulin after operation. Three patients required more insulin, and 2 were required to start insulin.

TABLE 6. CHANGES IN CARBOHYDRATE TOLERANCE

Average blood sugar before operation	164.3
Average blood sugar after operation	135.1
Insulin Requirements	
Number of patients	No.
Requiring insulin before operation	25
Requiring insulin after operation	19
Discontinuing insulin after operation	8
Requiring less insulin after operation	13
Starting insulin after operation	3
Requiring more insulin after operation	3
Requiring no insulin before or after operation	8

Respiratory quotient before and after operation. Figure 5 depicts graphically the change in the respiratory quotient before and after thyroidectomy. The basal rates were done by the old Tissot gas analysis method. It is probably safe to assume that the carbohydrate metabolism is better balanced when the respiratory quotient is in the higher group. This was true after operation in the large majority of instances, especially when the original quotient was low. The assumption is that, after thyroidectomy, a better storage of glycogen in the liver prevailed, and is further indirect evidence of an improved carbohydrate metabolism.

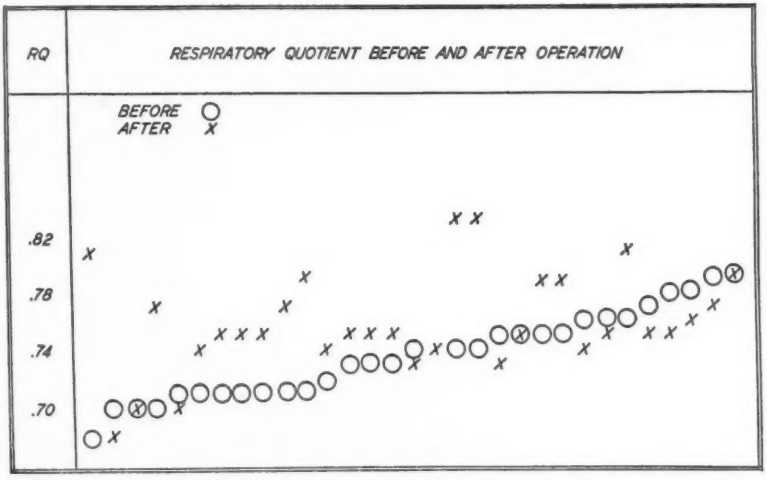


Fig. 5. Respiratory quotient before and after operation.

Type of operation. Over as long a period as this study covers (1925-1938), it is not surprising to note that all types of standard surgical procedures were used. Most of the surgery was done by Dr. Roy McClure. Two of the early cases first received x-ray therapy. This was done at the request of the patient in both instances, with little hope of success, and operation was later necessary. These patients, like many others with hyperthyroidism, were operation-shy. Joslin and Lahey attribute their ever-shrinking operative deaths to multiple-stage operations. They use ligation frequently. Two patients in this

series had ligations. Forty-two subtotal thyroidectomies were done, and one of these was done in two stages. One patient, operated upon elsewhere, was suffering a recurrence, and one of our own cases came back to the hospital with a recurrence (B.M.R. +24 per cent) and acidosis.

The operative mortality rate of the 42 cases is approximately 2.4 per cent. One patient died, two days after operation, of auricular thrombi and pneumonia (autopsy).

Care before and after operation. Approximately 10 days were required to prepare a thyrodiabetic of average severity, and otherwise uncomplicated,

TABLE 7. TYPE OF OPERATION

Preoperative x-ray therapy	2
Ligations	2
Subtotal thyroidectomy	41
Subtotal thyroidectomy—two-stage	1
Previous thyroidectomy	1[1]
Recurrent hyperthyroidism	1[2]

[1] Hyperplastic type—2 previous thyroidectomies.
[2] Acidosis 1 year after operation—B.M.R. +24%.

for operation. Diet prescriptions are detailed elsewhere, but the principle involved is to overfeed the patient and allow liberal amounts of carbohydrate, both of which favor glycogen storage in the liver. Insulin is used in amounts sufficient to bring the fasting blood-sugar to 125 to 150 mg. per 100 cc. of blood, and to render the urine sugar free or nearly so. Freedom from acidosis is very important and operation should not, in our judgment, be attempted if the blood CO_2 combining power is below 45 per cent. Hypoglycemia is to be avoided, for this drains glycogen from the liver. Divided doses of old insulin seem, so far, to be preferable to slowly acting insulins, as the changes in dosage necessary to meet the usual rapid changes in tolerance are more quickly and effectively made. Usually, single doses were held to 15 U.

TABLE 8. AVERAGE DIETS BY YEARS

Year	Diet		
	Protein	Fat	CHO
1925	51	140	63
1926	58	161	76
1927	60	200	80
1928	60	185	97
1929	62	160	100
1930	57	166	98
1931	61	153	123
1934	61	103	153
1935	67	201	212
1936	70	220	250

Electrocardiographic tracings are routine because of the tendency for hyperthyroid patients to have heart muscle changes. Also, for the protection of the surgeon, it is well to know that the vocal cords move normally before surgery is attempted. Beside diet and insulin orders, usual preoperative thyroid orders are indicated. Iodine in the form of Lugol's solution produces optimum lowering of the B.M.R. in from 7 to 10 days.

Glucose (400 cc. 10 per cent), intravenously, and old insulin subcutaneously (20 U) is administered before and after operation, and care is exercised after operation to make up the glucose, equivalent of the diet not eaten, with orange juice or actual glucose. Fluids to permit at least 1000 cc. of urine every 24 hours is important. Lugol's solution (drops X t.i.d.) is continued for approximately 3 weeks after operation. Ethylene and oxygen proved a satisfactory anesthetic, and at times ether was added to the mixture.

Average diets by years. During the period of this study, there has been a trend in diabetic diets which would make a general diet average misleading. Diets higher in carbohydrate have become more common in most clinics of late.

Our practice, at the present time, is to prescribe no diets containing less than 100 gm. of carbohydrate as such. This is increased to 200 gm. when a patient is being prepared for operation. In hyperthyroidism, this may even be increased to 250 gm., inasmuch at it is known that the patient with increased metabolism has a liver low in glycogen. The margins of safety seem wide, however, for the average diets actually used in this series varied from a carbohydrate amount of 63 gm. in 1925 to 250 gm. in 1936. The protein and fat amounts have also been increased to better meet the nitrogen and caloric needs of this type of patient.

SUMMARY

A group of 42 thyrodiabetics was studied and a total of 45 thyroidectomies were done. Cases were gleaned from 1616 diabetics and 1607 patients who had thyroidectomy. The incidence of thyrodiabetics to diabetic admissions was 2.41 per cent, and of hyperthyroid admissions 2.43 per cent. The highest incidence occurred in patients 51 to 70 years of age. Females predominated and 25 patients were born or lived in goiter regions before puberty. Nine cases have died and had an average survival of 4 years and 1 month. Vascular disease was a primary or complicating factor in all the deaths. Sixty four per cent had toxic adenoma. Evidence is presented which would indicate that, following thyroidectomy, the carbohydrate metabolism was improved. The operative mortality rate was 2.38 per cent. Preoperative and postoperative care is discussed.

The diagnosis of hyperthyroidism as a complication of diabetes mellitus, we feel sure, is being overlooked. When hyperthyroidism is complicating diabetes mellitus, thyroidectomy is indicated. Vascular disease associated with hypertension, since insulin has been available, constitutes the major cause of death in this group.

Acidosis is common but can be successfully combated by a high carbohydrate diet and adequate insulin.

REFERENCES

1. Wilder, R. M.: Arch. Int. Med. **36**:736. 1926.
2. Fitz, R.: Arch. Int. Med. **27**:305. 1921.
3. Joslin, E. P., and F. H. Lahey: Ann. Surgery **100**:629. 1934.
4. John, H. J.: Ann. Surgery **87**:37. 1928.
5. Ginsberg, A. M.: Endocrinology **15**:225. 1931.
6. Wilder, R. M., R. F. Foster and J. de J. Pemberton: Endocrinology **18**:455. 1935.
7. Joslin, E. P.: Treatment of Diabetes Mellitus; Lea & Febiger, Philadelphia, 1935.
8. McClure, R. D.: J.A.M.A. **109**:782, 1937.

A METHOD OF ASSAYING THE POTENCY OF ANTERIOR PITUITARY EXTRACTS WHICH INCREASE LIVER FAT

JAMES CAMPBELL
From the Departments of Physiology and Physiological Hygiene, University of Toronto
TORONTO, CANADA

It was observed by Best and Campbell (1) that anterior pituitary extracts were capable of producing an increase in the liver fat of fasting rats. In order to study the purification and properties of the active material from the glands it was decided to seek a suitable method of assay. Further work showed that, for this purpose, mice were more suitable than guinea pigs, rats or chickens (2). In the present paper a mouse assay of the substance or substances which are present in anterior pituitary extracts and which cause upon injection an increase in liver fat is described. The coincident effects of the extracts on liver glycogen and the resistance to insulin are also shown.

METHODS

The term 'liver fat' refers to the amount of fatty acids plus unsaponifiable fats determined by the direct saponification method described previously. Determinations of the fat in a sample of minced beef liver by 3 independent workers in the laboratory showed an error of $\pm$ 5 per cent in the method. This error in the chemical determination is not large in comparison with the variations encountered in the test animals. When liver glycogen was determined, the mice were anesthetized with sodium amytal before excision of the liver, and after the precipitation and washing of the liver glycogen the supernatant fluids were used for fat estimations.

The anterior pituitary extract was prepared as described previously (1) for studies on rats. It is obtained in the form of a dry powder which is stable for long periods when kept at 7°C. This extract is referred to as the 'A.P.P.' All injections were given subcutaneously, and in the latter experiments the volume per injection was kept fixed at 0.4 cc., variations in dose being produced by dilution of the extract with saline.

The test animals should be in as good condition as possible, free from parasites and with ample stores of depot fat. Since Coope and Mottram (3) found that an increase in liver fat may occur during pregnancy, when female mice are used they should be non-pregnant. During the test the animals were kept in a room at 23°C.

EXPERIMENTAL RESULTS

From previous work (2) it was apparent that, following the administration of A.P.P. to fasting mice, a rapid and extensive increase in the fat content

of the liver occurred. In 7 hours the liver fat had increased approximately threefold. As the time elapsed after the injection of A.P.P. the liver fat continued to increase, but the values became more variable and the liver fat of the fasting (control) animals also increased. The advantage of an increased effect, due to a period longer than 7 hours, seemed therefore to be reduced by

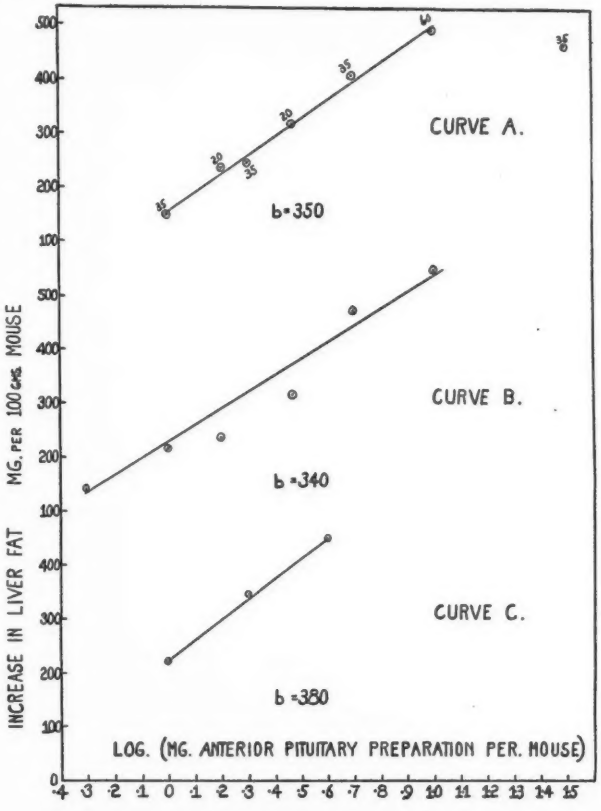


Fig. 1. Female mice, 7-hour period. In curve *A* the figures denote the number of mice used to determine the points.

the fasting increase in liver fat and the greater variability in the results. The nature of this response to the A.P.P. has been studied at 7-hour and 16-hour periods.

Response of female mice in a 7-hour period. The increases in the liver fat of female mice determined 7 hours after the injection of varying doses of A.P.P. are shown in figure 1, curves *A*, *B* and *C*. The data of 5 experiments performed at the beginning of this work have been grouped together to give curve *A*. In the separate experiments comprising this curve, usually 2 groups of five mice were injected with a single dose of preparation, 5 doses within the range 0.5 to 10 mg. being given. The control mice received saline injections.

The liver fat of each group of 5 mice was determined. In 3 of the experiments an 'ordinary strain,' in the other two, a 'Webster strain' of mice were used.

The data of figure 1, curve *B*, were obtained from a single experiment. Six doses of A.P.P. were given, and for each dose 4 groups of 5 mice were used,

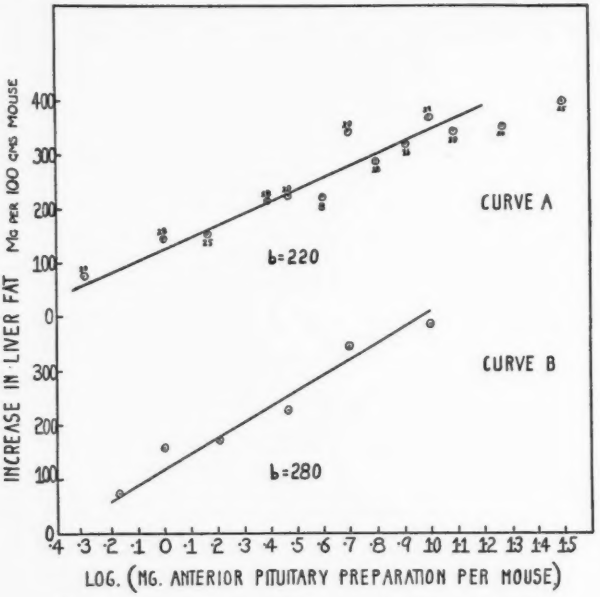


Fig. 2. Male mice, 7-hour period. In curve *A* the figures denote the number of mice used to determine the points.

TABLE 1. FEMALE MICE. THE LIVER FAT DETERMINED IN GROUPS OF 5. EXPERIMENTAL PERIOD, 7 HOURS

No. of Mice	Avg. Wt. gm.	Ant. Pit. Preparation per mouse[1] mg.	Liver fat per 100 gm. mouse mg.	Increase in liver fat per 100 gm. mouse mg.	Std. Dev. σ	$\sigma_{t.m.}$ from smoothed curve $n=20$.
20	23.8	10.0	798	552	135	30.2
20	23.4	5.0	722	476	168	39.2
20	24.5	3.0	561	315	102	44.7
20	23.5	1.6	485	239	40.9	30.7
20	23.2	1.0	465	219	88.3	19.8
10	23.6	0.5	387	141		
15	24.4	Saline	246	0		

[1] Following the injection of anterior pituitary preparation or saline, the mice were fasted.

except for the lowest dose for which 2 groups of 5 were used (table 1). The liver fat of each group of 5 mice was determined. In another experiment (fig. 1, curve *C*) the number of animals for each dose was increased to 30, three doses were given and the liver fat of individual mice was determined (table 2). 'Cullen' mice were used for curves *B* and *C*. The experiments of curves *A*,

B and *C* were carried out in May and June, November and December respectively.

We shall comment later on the reasons for plotting the response against the logarithm of the dose. From figure 1, it is considered that for practical purposes of assay these curves are approximately straight lines between the limits 0.5 to 10 mg. A.P.P. per female mouse. Above this range further increases in dose have no greater effect on the liver fat, and below this dosage—a region which has not been investigated carefully—the values seem to be erratic and tend to be high. The slopes of the curves are found by the formula

$$b = \frac{d\ (\text{Increase in liver fat})}{d\ (\log \text{dose})}$$

the liver fat being expressed as mg. per 100 gm. mouse and the dose as mg. of A.P.P. per mouse. The slopes of these curves are quite uniform (b of curve $A = 350$; curve $B = 340$; curve $C = 380$) and if each value of b is given the same weight the average is $b = 357$.

Response of male mice in a 7-hour period. The responses of male mice 7 hours after the injection of varying doses of A.P.P. are shown in figure 2, curves *A* and *B*. Curve *A* is a composite of the data of 3 experiments, 2 with an 'ordinary strain' and one with 'Webster strain' mice, the procedure being similar to that used in the first experiments on female mice (fig. 1, curve *A*). The data of figure 2, curve *B* (also table 3) were obtained from a group of male 'Webster' mice in an experiment similar to that of figure 1, curve *B*.

TABLE 2. FEMALE MICE. THE LIVER FAT DETERMINED IN INDIVIDUAL MICE. EXPERIMENTAL PERIOD, 7 HOURS

No. of Mice	Avg. Wt. gm.	Ant. Pit. Preparation per mouse[1] mg.	Liver fat per 100 gm. mouse mg.	Increase in liver fat per 100 gm. mouse mg.	Std. Dev. σ	$\sigma_{t.m.}$ from smoothed curve $n=20$
30	18.6	4.0	703	450	163	36.4
30	18.3	2.0	596	343	132	29.5
30	18.6	1.0	473	220	105	23.6
8	18.2	Saline	253	0	23.2	

[1] Following the injection of anterior pituitary preparation or saline, the mice were fasted.

The experiments relating to curves *A* and *B* were performed in May and June and in October respectively.

The points of the 'log dose—response' curves for male mice under these conditions also appear to be fitted best with straight lines between the limits of 0.5 to 10 mg. of A.P.P. per mouse. The values of the slope b of curves *A* and *B* are 220 and 280 respectively, giving an average of 250. From this it follows that the ratio of the response of female mice to that of male mice was 357/250 or 1.4/1. In other words, after a dose of A.P.P. under these conditions the extent of the increase in liver fat of the females was 1.4 times greater than that of the males.

Response of female mice in a 16-hour period. The nature and variation of

the responses in female mice 16 hours after the injection of extract are shown in figure 3, table 4. Groups of 23 fasting female mice were injected with varying doses of anterior pituitary preparation, and the fat in the individual mouse livers was determined. Under these conditions also a straight line seems to fit the points of the 'log dose—response' curve. The slope of the curve, $b = 640$, is much greater than the slope of the curve for female mice after a 7-hour period, in which case $b = 357$. It would be expected that this increased value of b should decrease the error of the test, but two factors operate against this. These factors are the high level of the control liver fat which reduces the percentage of increase in liver fat due to the anterior pituitary preparation, and the increased standard deviations of the responses. As will be shown in

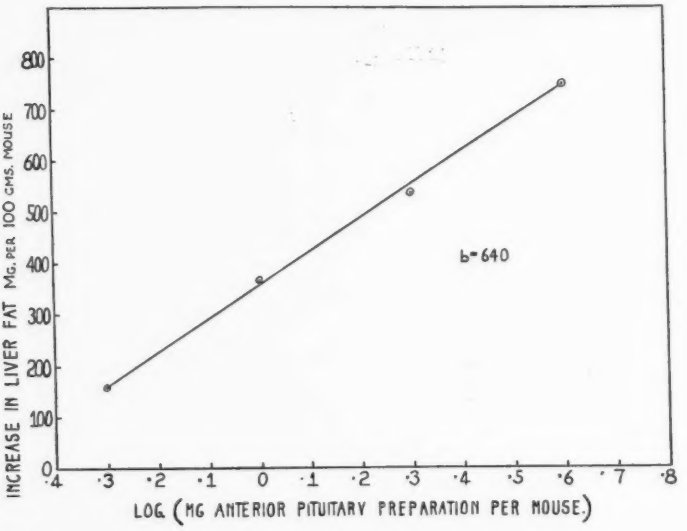


Fig. 3. Female mice, 16-hour period.

TABLE 3. MALE MICE. THE LIVER FAT DETERMINED IN GROUPS OF 5. EXPERIMENTAL PERIOD, 7 HOURS

No. of Mice	Avg. Wt. gm.	Ant. Pit. Preparation per mouse[1] mg.	Liver fat per 100 gm. mouse mg.	Increase in liver fat per 100 gm. mouse mg.	Std. Dev. σ	$\sigma_{t.m.}$ from smoothed curve $n=20$
15	20.4	10.0	631	389	16	17
20	20.8	5.0	587	345	55	15
20	20.9	3.0	465	223	87	35
20	20.6	1.6	411	169	79	25
20	20.6	1.0	397	155	26	10
10	20.0	0.5	319	77		
10	20.4	Saline	242	0		

[1] Following the injection of anterior pituitary preparation or saline, the mice were fasted.

TABLE 4. FEMALE MICE. THE LIVER FAT DETERMINED IN INDIVIDUAL MICE. EXPERIMENTAL PERIOD, 16 HOURS

No. of Mice	Avg. Wt. gm.	Ant. Pit Preparation per mouse[1] mg.	Liver fat per 100 gm. mouse mg.	Increase in liver fat per 100 gm. mouse mg.	Std. Dev. σ	$\sigma_{t.m.}$ from smoothed curve $n=20$
23	18.1	4.0	1354	749	292	65.5
23	18.9	2.0	1142	537	327	71.6
23	19.4	1.0	971	366	281	62.9
20	18.3	0.5	765	160	268	52.7
10	18.7	Saline	605	0	238	

[1] Following the injection of anterior pituitary preparation or saline, the mice were fasted.

the following section, the error of the test under these conditions differed only slightly from the error when a 7-hour period was used.

Variation of the responses. The interpretation of the variations encountered in this work has been directed by Dr. Mary A. Ross.[1] The approach to this problem has been practical, and certain assumptions have been made which can only be put to rigid test when much experimental evidence has accumulated. It would appear that within the dosage limits mentioned the 'log dose—response' curve is a straight line and that under the best conditions the slope is fairly constant. It is possible, however, to fit to the points obtained, curves which deviate from a straight line; and it is apparent that the slope may vary with less uniform animals.

Frequency curves constructed from the data of tables 2, 4, 5, and of other experiments showed that the frequency distributions of the responses were approximately normal. The standard deviations (σ) were found by the usual formula from the data of tables 2, 4, and 5. For the data of tables 1 and 3 rough approximations to the standard deviations (of the individual mice) were found by the formulae *1)* and *2)*, since in these experiments the fat contents of groups of 5 mouse livers were determined, 4 such groups (20 mice in all) being given a single dose of A.P.P.

1) $\sigma_g = \sqrt{\dfrac{\Sigma d_g^2}{n_g - 1}}$ where σ_g = the standard deviation of the groups of 5 mice from the mean.
n_g = the number of groups (4).
d_g = the deviations of the groups of 5 mice from the mean.

2) $\sigma = \sigma_g \times \sqrt{n}$ n = the number of mice in a group (5).

In the calculation of the standard deviations of the means, the number of mice for each value was considered to be 20, although the number of mice actually used may have differed from this (20 to 30 mice except for one group of 15).

If we consider the slope of the log dose—response curve for female mice with the 7-hour experimental period to have an average value of $b = 357$, a better indication of the deviation may be obtained by finding the deviation of the observed value from the values obtained from the smoothed curve of slope $b = 357$, but this does not take into account variations in the value of b. Similarly, for male mice the deviations from the curve of $b = 250$ were taken. The mean increases in liver fat obtained from the smoothed curves were called for convenience 'true means' and the standard deviations from the true means ($\sigma_{t.m.}$) were calculated.

[1] Of the Department of Epidemiology and Biometrics, School of Hygiene, University of Toronto.

TABLE 5. THE EFFECT OF TEMPERATURE
FASTING FEMALE MICE

Temperature	Ant. Pit. Preparation per mouse mg.	No. of Mice	Avg. Wt. gm.	Liver fat per 100 gm. mouse[1] mg.	Std. Dev. σ
37.2°C.	4.0	20	20.4	597	101
23.5°C.	4.0	20	20.4	609	109
4.0°C.	4.0	20	18.4	674	148
23.5°C	0 (saline)	8	19.2	342	69

[1] The fat was determined in individual mouse livers 3½ hours after the injection.

It was considered that the values of the standard deviations of the true means might be averaged. With 20 mice in a group, the data of tables 1 and 2 gave an average value of $\sigma_{t.m.} = 31.8$. From tables 3 and 4 the corresponding values were 20.5 and 63.2 respectively.

In a test of this nature, where the slope of the log dose—response curve is assumed constant and two equal groups of animals are injected, one with a standard dose and the other with an unknown dose of active material, the standard deviation (λ) of the ratio $\log \left\{ \frac{\text{potency of unknown}}{\text{potency of standard}} \right\}$ is expressed by formula

3) $\lambda = \pm \sqrt{2} \frac{\sigma_m}{b}$ when σ_m = the standard deviation of the mean
b = the slope

In this work $\sigma_{t.m.}$ is used instead of σ_m for the reason mentioned above. It is recommended in the British Pharmacopoeia Commission Report (4) that, in biological tests of this nature, the limits within which the results would fall in 99 cases out of 100 should be stated. Where the error is normally distributed these limits correspond to the range covered by ± 2.576 times the standard deviation.

In a 7-hour period, using 20 female mice per group, one group being injected with standard extract, and one with an unknown

$$\lambda = \pm \sqrt{2} \frac{31.8}{357} = \pm 0.126$$

In 99 cases out of 100, the limits of error in the determination of the log dose are therefore $\pm 0.126 \times 2.576 = \pm 0.324$.

If the true dose is equivalent to 1 mg. A.P.P. the limits of error in the deter-

TABLE 6. EFFECT OF DIVIDED DOSES
FASTING FEMALE MICE

Group[2]	No. of Mice	Avg. Wt. gm.	Total dose of ant. pit. preparation per mouse mg.	Liver fat per 100 gm.[1] mouse mg.	Std. Dev. σ
A (single dose)	24	19.1	4.0	771	144
B (single dose)	25	18.6	2.0	646	187
C (divided doses)	25	19.1	4.0	765	124
D (divided doses)	25	18.4	2.0	688	137

[1] The fat was determined in individual mouse livers 7 hours after the first injection of anterior pituitary preparation.
[2] Groups *A* and *B* received single doses of extract, groups *C* and *D* were injected at the beginning of the experiment and 3½ hours later.

TABLE 7. FASTED MALE MICE
EXPERIMENTAL PERIOD, 7 HOURS

No. of Mice	Avg. Wt. gm.	Injection	Dose per mouse mg.	Liver Glycogen		Liver Fat	
				per 100 gm. mouse mg.	%	per 100 gm. mouse mg.	%
9	27	A.P.P.	8.0	230	3.10	690	9.31
9	25	Saline	—	17	0.29	270	4.47

mination of the dose are therefore 2.1 mg., and 0.47 mg. If 30 mice per group are used, the limits are 1.8 and 0.55 mg. Similarly, in a 7-hour period with 20 male mice per group, the limits are 2.0 mg. and 0.5 mg., and in a 16-hour period with 20 female mice per group the limits are 2.3 mg. and 0.44 mg.

Variable factors. We have already briefly described the influences of the strain of mice, sex, season of the year and the length of the test period on the responses. Some other variable factors of the test have been considered.

Body weight. In examining the results of the series comprising the data of figure 1, curve *A,* it was noted that in female mice fasted for 7 hours (controls) the amount of fat in the liver increased as the body weight increased. When the control liver fat was plotted against body weight, it seemed that in 100 gm. mouse, for every increase of 1 gm. in body weight, an increase of 16 mg. liver fat occurred, but it is not suggested that this is a reliable value. It is advisable to restrict the weight of the mice used in the assay to under 24 gm., in order to reduce the high control liver fat level. It might be expected from this that heavy female mice would respond more to the anterior pituitary injections than lighter ones. From the data of tables 2, 4, and 5, and other experiments, the responses of the individual mice were plotted against body weight, but in a total of 360 female mice no definite relation between body weight and response to the A.P.P. could be seen.

In male mice the relation between the control liver fat level and body weight is not the same as in female mice. In the experiments comprising the data of figure 2, curve *A,* the control liver-fat values (after 7 hours of fast) remained at the same level within the limits of body weight investigated (18 to 38 gm.). This difference in the sexes may be related to the finding that female mice give a greater response to the anterior pituitary injections than males.

TABLE 8. FASTING MALE MICE. 10 PER GROUP

A.P.P. dose per mouse mg.	Liver fat per 100 gm. body wt. mg.	Ratios of insulin doses[1]	Convulsions %
10	825	7.3	100
10	772	5.5	60
10	750	3.1	50
10	790	1.7	30
0 (saline)	306	1.0	90

[1] The insulin test was performed 6 hours after the injection of A.P.P.

Temperature. Trevan (5) showed that the control of temperature was important in the assay of insulin by the convulsions produced in mice. The effect of temperature on the increase in liver fat produced by the anterior pituitary extract is shown in table 5. Sixty mice injected with the same dose of A.P.P. (4 mg. per mouse) were separated into 3 equal groups, one being kept in a room at 4°C., another at 23.5°C., and another in the apparatus used for the mouse assay of insulin (6) at 37.2°C. A group of 8 mice were injected with saline and kept at 23.5°C. The mice were distributed 4 in a cage, and after injection and removal of food the liver fat was determined in individual mice 3½ hours later, as it was not considered advisable to keep the mice at 37°C. longer than this.

The effect of the A.P.P. on the liver fat did not increase with temperature. Rather, there appeared to be an increase as the temperature was lowered, although these differences were small. It appears, therefore, that the error of the test cannot be greatly affected by a change in slope due to temperature.

Divided doses. The effect of giving the A.P.P. to female mice in 2 doses instead of 1 in a period of 7 hours was investigated (table 6). Four groups of 25 mice were used, two groups being given injections at 0 and 3½ hours of doses totalling 4 and 2 mg. A.P.P. The other two groups were given the same total doses in one injection at time 0. The mice were fasted for 2 hours previous to injection and, as usual, following the injections. By dividing the doses, no significant increase in the extent of the response or of the slope of the curve occurred. The standard deviations may be reduced when the doses are divided, but it is doubtful if the improvement (if any) would repay the additional handling of the animals.

The effect of the A.P.P. on liver glycogen. The effect of the A.P.P. on the liver glycogen of fasting mice coincident with the effect on liver fat is shown in a typical experiment (table 7).

The mice were fasted following injection, and the liver glycogen and fat determined after 7 hours. The effect on liver glycogen was even more extensive than the effect on liver fat. Mice would therefore appear to be suitable animals for tests of the 'glycostatic' activity of anterior pituitary extracts under the same conditions as used for the 'liver fat' test.

Effect of the A.P.P. on insulin resistance. Under the same conditions as in the above experiment, the A.P.P. develops in mice a considerable resistance to the convulsions produced by the injection of insulin. The results of a representative experiment are shown in table 8. The mice were injected with A.P.P. or saline (controls) and fasted. After 6 hours the number of convulsions produced by graded doses of insulin were determined, using the apparatus of Trevan and Boock at a temperature of 37°C. The A.P.P. produced such a resistance to insulin that between 5.5 and 7.3 times the dose of insulin was necessary to produce the same number of convulsions in the mice which had received 10 mg. A.P.P. as in the controls.

DISCUSSION

The experiments show that it is possible to estimate the activity of extracts of the anterior pituitary gland which increase liver fat by the use of normal

mice. For assay it would appear best to administer single doses of unknown and standard extract and to determine the liver fat 7 hours later, the animals being fasted during this period. In this work the slopes of the log dose—response curves under a given set of conditions were regular, so that it may be permissible to use, with relatively uniform animals, only one group injected with unknown solution and one with standard. If the responses are not equal, the relative potency of the unknown to standard may then be obtained by reference to the standard curve or by calculation. This matter of variation in the slope (b) of the log dose—response curve might, however, be left to the experience of the operator. The number of animals used and the number of tests should depend on the accuracy desired.

The level of fat in the livers of mice after the injection of anterior pituitary extract increased slightly as the temperature was lowered over a wide range. This result may be related to the observation (7) that low temperatures produce a rapid infiltration of fat in the livers of mice. It may be noted that since the number of convulsions produced in mice by the injection of insulin are greatly increased by a rise in temperature (5), a fundamental difference in the mode of action of the two substances is indicated.

It was observed by Houssay, Mazzocco and Rietti (8) that hypophysectomy caused a diminution in the blood sugar and liver glycogen of toads, and Russell (9) has described a test for the 'glycostatic' activity of anterior pituitary extracts by the use of hypophysectomized rats. It is indicated that normal mice, under the conditions of the 'liver fat' test are also suitable test animals for glycostatic activity. It was also found that the A.P.P. produced a 'glycotropic' as well as a 'glycostatic' effect, for when the liver glycogen was first depleted by a period of fast (9 hours) the injection of A.P.P. caused in the course of 6 hours an increase of liver glycogen over the control level. It is of interest that the A.P.P. caused a simultaneous increase in liver fat and liver glycogen over the control level in fasting mice. This observation is contrary to the theory of Rosenfeld (10) that an antagonism exists between fat and carbohydrate in the liver.

We have found in several experiments that a short time after the injection of A.P.P. (6 hours) the dose of insulin necessary to produce convulsions in the injected mice is much greater than in the controls. It is indicated that mice would be suitable animals with which to test this property of anterior pituitary extracts, using approximately the same conditions as are used in the 'liver fat' test.

These tests, particularly the liver fat test, have been used for some time in these laboratories, and the investigations on the fractionation of anterior pituitary extracts will be reported later.

SUMMARY

A method of determining the potency of anterior pituitary extracts in increasing liver fat by the use of normal mice is described. Female mice were more sensitive than males to the anterior pituitary preparation by the proportion of 1.4 to 1. As the temperature was lowered from 37°C. to 4°C., the

amount of fat in the liver after the injection of anterior pituitary preparation was slightly increased. Coincidentally with the increase in liver fat the anterior pituitary extract caused an increase in liver glycogen and a resistance to insulin.

My sincere thanks are due to Professor C. H. Best, who directed this work, and to Dr. Mary A. Ross of the Department of Epidemiology and Biometrics, School of Hygiene, who supervised the statistical treatment of the results. It is a pleasure to thank Miss Ann Jaffray for technical assistance.

REFERENCES

1. Best, C. H., and J. Campbell: J. Physiol. **86:**190. 1936.
2. Best, C. H., and J. Campbell: J. Physiol. **92:**91. 1938.
3. Coope, R., and V. H. Mottram: J. Physiol. **49:**23. 1914.
4. British Pharmacopoeia Commission Report No. 10. 1936.
5. Trevan, J. W.: Proc. Roy. Soc. London, s. B. **101:**483. 1927.
6. Trevan, J. W., and E. Boock: Document C.H. 398, Health Organization of the League of Nations. 1926.
7. Lanczos, Anna: Arch. f.d. ges. Physiol. **235:**422. 1935.
8. Houssay, B. A., P. Mazzocco and C. T. Rietti: Compt. rend. Soc. de biol. **93:**967. 1925.
9. Russell, J. A.: Endocrinology **22:**80. 1938.
10. Rosenfeld, G.; Ergebn. d. Physiol. **1:**50. 1903; Arch. f. exper. Path. u. Pharmakol. **166:**211. 1932.

AN EVALUATION OF THE FRANK METHOD FOR THE DETERMINATION OF PROLAN (GONADOTROPIC PRINCIPLE) IN THE URINE OF NONPREGNANT WOMEN[1]

DELLA G. DRIPS AND ARNOLD E. OSTERBERG

WITH THE TECHNICAL ASSISTANCE OF GERTRUDE FISHER AND KATHLEEN LEWIS

From the Section on Obstetrics and Gynecology and the Section on Clinical Biochemistry, The Mayo Clinic

ROCHESTER, MINNESOTA

For some time we have been seeking a quantitative method for determining prolan in the urine of nonpregnant women when it is present in amounts of less than 66 R.U. per liter. The Aschheim-Zondek alcohol precipitation method (1), which employs 60 cc. of morning urine, does very well when amounts of prolan in the urine are in excess of 66 R.U.

We found that many women suffering from vasomotor disturbances of the menopause did not have sufficient prolan in 60 cc. of urine to give a positive test; yet we felt that their urinary prolan must be in excess of normal. It is not so important to have a method for the detection of excess prolan in the urine of women who are able to describe typical vasomotor waves, but in cases of those suffering from some bizarre syndrome or psychosis, or those whose vasomotor waves have persisted for years after the cessation of the menses, it is desirable to have a method which will show whether or not the gonadotropic hormone of the anterior pituitary lobe is being secreted in excessive amounts.

We were also very desirous of obtaining a measure of pituitary-gonadotropic function in the case of younger women who had functional menstrual irregularities, sterility and associated complaints. This meant that we must detect small amounts of prolan, since we had found that several apparently normally menstruating women had at no time in the cycle amounts which we could detect by any method. We had tried several methods for determining small amounts, including those advocated by Katzman and Doisy (2), and Levin and Tyndale (3). We wanted to have a reliable measure for small normal amounts of prolan in order to know what could be considered an excess amount, as this is assumed to be an evidence that ovarian failure of some degree has occurred. On the other hand, it has been assumed that if not even small amounts of prolan could be demonstrated by several determinations over a period of time we were dealing with decreased pituitary function in relation at least to the secretion of the gonadotropic hormone.

When we reviewed the method reported by Frank, Salmon and Friedman (4), we felt it might provide us with a reliable means for determining any quantity of prolan in the urine of nonpregnant women. It is a modification

[1] Read at the meeting of the American Physiological Society, Baltimore, Maryland, March 30, 1938.

of the Aschheim-Zondek method, and uses acetone instead of alcohol, as acetone makes a more stable precipitant. Instead of beginning with 60 cc. of urine, 400 cc. is used, and in the final amount for biologic assay (8 cc.) 2 cc. represents 100 cc. of urine.

In making the biologic assay, varying amounts of the extract are injected into immature animals in a series of injections over 2 or 3 days, as is usual; the smallest amount of injected material which gives a positive reaction represents the largest amount of prolan present in the urine, and vice versa. For example, when a positive reaction is obtained with 0.2 cc. of the extract, this represents 100 R.U. to a liter of urine; a positive reaction with 2 cc. of extract represents 10 R.U., and, with 4 cc., 5 R.U. to a liter of urine.

Albino rats of the Wistar strain were used. The uteri and ovaries were observed grossly and weighed, some at the end of 72 hours; the majority, however, were weighed at the end of 96 hours. The ovaries were then fixed in Bouin's fluid, embedded in paraffin, cut and stained in the usual manner, and observed for evidences of follicle stimulation and luteinization.

TABLE 1. MENOPAUSE FOLLOWING CESSATION OF PERIODS

Spontaneous menopause					Artificial menopause					Prolan not demonstrated
Case	Hot flushes, grade	Prolan positive, excess amounts, R.U.	Prolan negative, excess amounts, R.U.	Prolan positive, normal amounts, R.U.	Case	Hot flushes, grade	Prolan positive, excess amounts, R.U.	Prolan negative, excess amounts, R.U.	Prolan positive, normal amounts, R.U.	
1	3	66			1[3]	0			20	
2	3	66			2[3]	0			20	
3	2	66			3[4]	0			20	
4	2	45			4[5]	1	40			
5	3	25			5	0				0
6	1		<25[1]		6	2	66			
7	3			20	7	2	66			
8	3	40			8	1?				0
9	2	40			9	3	40			
10	2			5	10	2			20	
11	1	25			11	0		<exc.		
12	3		<20		12[3]	2	40		20	
13	0			20	13	2				0
14	2	66			14	0		<exc.		
15	1			20	15	1			20	
16	0	40			16[3]	2	45			
17	3	66			17	3	66			
18	3	66								
19	1		<exc.[2]							
20	1			5						

[1] Negative for 25 R.U.
[2] Negative for excess amounts.
[3] Panhysterectomy; transplantation of one ovary.
[4] X-ray and hysterectomy.
[5] Transplantation of one ovary.

After reviewing the Levin and Tyndale (5) method of assaying prolan in mice by uterine weights, we felt it might be possible to use the weight of the uterus in rats as a criterion for the amounts of prolan present. Levin found that the uterus of the mouse shows more change at the end of 72 hr. than at the usual period of 96 hr. following the first injection. As we have always

weighed the uterus and ovaries of experimental and control animals at necropsy, we had much data on uterine weights of normal animals for certain ages and body weights. With this particular method of extracting prolan we have found a definite increase in the weight of the uterus in certain litters of animals when they were examined at necropsy at the end of 96 hr. On observing their ovaries

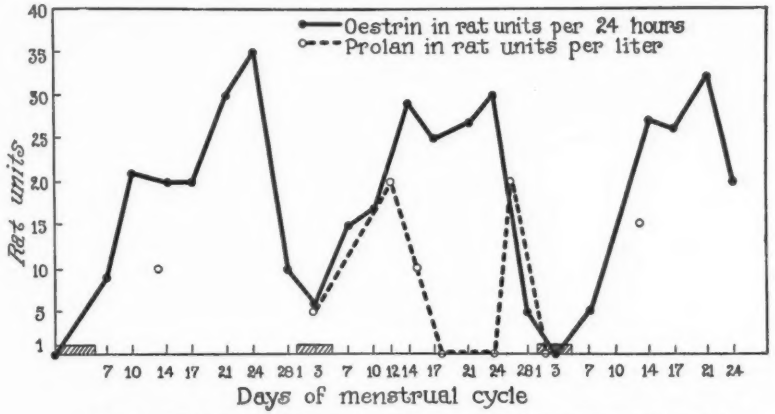


Fig. 1. Graph showing the values for estrin and prolan during the menstrual cycle of a young woman with regular and normal menses.

microscopically, we found them to contain one or two very large well-developed follicles; the other follicles were small and undeveloped, as though these one or two follicles had developed at the expense of the others. In other ovaries of animals receiving injections there was a more or less uniform increase in the number and size of follicles throughout the whole ovary. This we considered just as significant a positive finding, as far as the ovaries were concerned, although the uterine weights had not been changed. It is our belief, therefore, that if dependence is placed on uterine weights only, certain positive results will be missed.

The same conclusion seems to hold true for animals killed at 72 hr., although as yet we do not have a sufficiently large number of experiments to draw accurate conclusions. There does seem to be an even greater increase in uterine weight at 72 than at 96 hr. in some litters of animals, but the microscopic changes in the ovaries are more reliable than the weights of the uterus, and the microscopic changes in the ovaries at 96 hr. seem more definite and reliable than those at 72 hr.

The weights of the ovaries of animals that have been given injections of nonpregnancy urine extracts change very little, and cannot be relied on. There is a marked change in weight when corpora lutea develop, but this seldom occurs with this concentration of urine.

A definite increase in the weight of the uterus lends support to a positive ovarian response, but, as has been said, we have found that the microscopic changes in the ovaries at 96 hr. are the most reliable criterion for a quantitative estimation of the urinary prolan. These microscopic changes consist of evidences

of follicle stimulation and, very occasionally, of luteinization. The corpora lutea, when present, appear as normally formed structures.

In order not to use too many small rats in each assay, clinicians are in the habit of trying to estimate in what place in the scale we expect the prolan estimation to come. For instance, in the case of women in the menopause we are interested only in excess amounts of prolan, 20-66 R.U. or more to a liter. When we suspect pituitary failure, we are interested only in knowing whether small normal amounts are present, 5, 10 or 15 R.U. to a liter. When we suspect mild or partial ovarian failure in young women, we are interested in amounts slightly in excess of normal, 20, 25, or 40 R.U. If these are present, we then test for higher amounts or vice versa. Litter mates are used, and one rat of

TABLE 2. FUNCTIONAL MENSTRUAL DISTURBANCES

Clinical diagnosis	Cases	Prolan: positive excess amounts	Prolan: positive normal amounts	Prolan not demonstrated
A. Primary amenorrhea:				
Primary ovarian	6		3	3
Secondary ovarian	1			1
B. Secondary amenorrhea:				
Primary ovarian	12	1	6	5
Secondary ovarian	29		9	20
C. Ovarian dysfunction of amenorrheic type:				
Primary ovarian	53	15	25	13
Secondary ovarian	36		6	30
D. Ovarian dysfunction of metrorrhagic type:				
Primary ovarian	10		10	
Secondary ovarian	11		1	10
Totals	158	16	60	82

each litter is kept as the control. A card from the clinician to the technician to test for these amounts of prolan goes with each specimen. The technicians who make the biologic assay therefore know nothing more than this about the case. I believe this is important, for when one observes follicle stimulation in the ovaries, which is often the only positive end-point for the small amounts, a clinician might be biased by his knowledge of the case.

It was decided to test the Frank method first by using it for women who were definitely in the menopause, and who complained of hot flushes (table 1). This shows how the more severe flushes occur in cases in which greater amounts of excess prolan are present in the urine. A few women complain of flushes who have only 20 R.U. of prolan per liter of urine. In only two cases in this group in which patients complained of flushes were we unable to demonstrate prolan with one test.

We have found that it is not always possible to demonstrate prolan in the urine of normally menstruating women by this method. The time when prolan is present varies considerably in women with regular, apparently normal

menses, normal curves for estrin, and normal premenstrual endometrium. Prolan is probably most often present on the 13th day of the menstrual cycle, although occasionally it is found only just before or during the menses. It

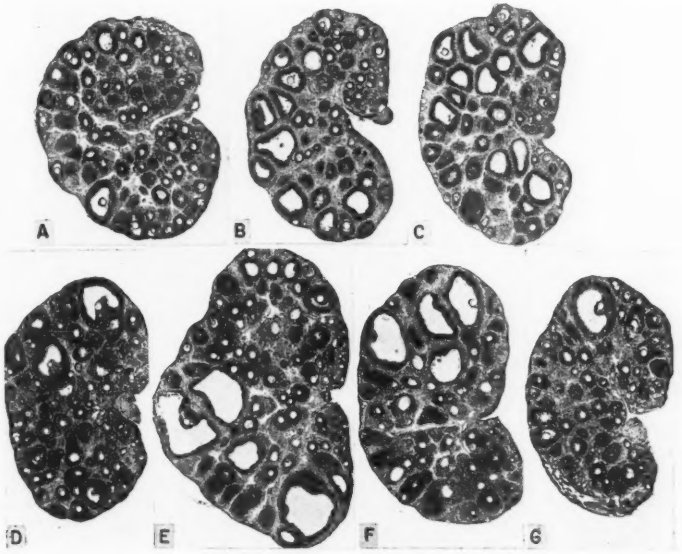


Fig. 2. *Upper row:* Ovarian response to injections of urine extract containing gonadotropic substance in the case of a young woman with functional menstrual irregularity. *A*, Control. *B*, Experiment 1, positive. *C*, Experiment 2, positive. CASE 1.

Case 1: 20-day old litter mates
Positive for 10 rat units, negative for higher amounts.

Extract injected	Weight of ovaries, mg.		Weight of uterus, mg.
Control	4.5	4.5	20
Exp. 1: 4 cc.	4.5	5.0	18 (5 R.U.)
Exp. 2: 2 cc.	3.5	3.0	19 (10 R.U.)

Lower row: Ovarian response to injections of urine extract containing gonadotropic substance in the case of a woman, 35 years of age, with a depressive psychosis and oligomenorrhea. *D*, Control. *E*, Experiment 1, positive. *F*, Experiment 2, positive. *G*, Experiment 3, positive. CASE 4.

Case 4: 20-day old litter mates
Strongly positive for 66 rat units

Extract injected	Weight of ovaries, mg.		Weight of uterus, mg.
Control	5.0	4.0	23
Exp. 1: 0.8 cc.	5.0	7.5	112 (25 R.U.)
Exp. 2: 0.44 cc.	5.0	5.0	88 (45 R.U.)
Exp. 3: 0.3 cc.	5.0	4.5	75 (66 R.U.)

varies in amount from 5 to 15 R.U. per liter. We believe that up to 20 R.U. may be considered normal. When prolan is present in the urine in amounts greater than 20 R.U., the women may at any age present menstrual irregularities and associated clinical symptoms. Vasomotor disturbances may even be present.

These women are suffering from ovarian failure which may be of any degree of severity.

Over many years of diagnostic study in cases of functional menstrual

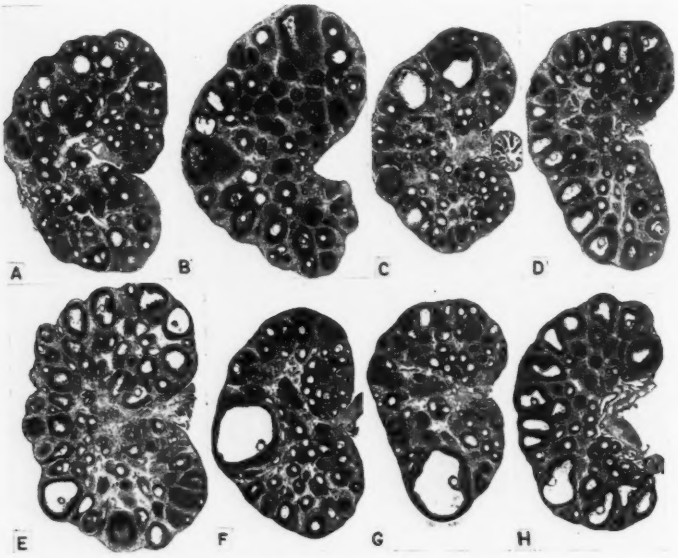


Fig. 3. *Upper row.* Ovarian response to injections of urine extract containing gonadotropic substance in the case of a young woman with a functional menstrual irregularity. *A,* Control. *B,* Experiment 1, positive. *C,* Experiment 2, positive. *D,* Experiment 3, negative. CASE 5.

Case 5: 23-day old litter mates[1]
Prolan-A positive for 10 R.U., Frolan-B positive for 5 R.U.

Extract injected	Weight of ovaries, mg.		Weight of uterus, mg.
Control	4.0	3.5	18.0
Exp. 1: 4 cc.	8.0	8.0	96.0 (5 R.U.)
Exp. 2: 2 cc.	3.0	3.0	31.0 (10 R.U.)
Exp. 3: 1 cc.	4.5	4.0	16.5

[1] Same body weight as 21-day old.

Lower row. Ovarian response to injections of urine extract containing gonadotropic substance in the case of a girl, 15 years of age, with undeveloped genitalia and breasts, who has never menstruated. *E,* Control. *F,* Experiment 1, positive. *G,* Experiment 2, positive. *H,* Experiment 3, positive. CASE 6.

Case 6: 21-day old litter mates
Positive for 20 rat units

Extract injected	Weight of ovaries, mg.		Weight of uterus, mg.
Control	6.0	6.0	24
Exp. 1: 4 cc.	3.5	3.5	124 (5 R.U.)
Exp. 2: 2 cc.	3.0	4.0	73 (10 R.U.)
Exp. 3: 1 cc.	6.0	6.0	20 (20 R.U.)

disturbances (6), we have acquired certain clinical and laboratory evidences of dysfunction, which place patients into two groups: *a),* those with apparent primary ovarian failure of varying degrees, and *b),* those with secondary

ovarian failure. Patients with primary partial ovarian failure, in addition to menstrual irregularity, have many complaints associated with their irregular and abnormal periods, such as soreness of the breasts and increased nervousness prior to the periods, dysmenorrhea, menstrual migraine and, occasionally, menstrual urticaria and edema and acne. They usually have normal basal metabolic rates, and when in such cases we obtain evidence of normal pituitary function, we have the final proof that the dysfunction is ovarian. When more severe ovarian failure is present the pituitary-gonadotropic function apparently becomes more stimulated, the amounts of prolan become excessive, and these women present vasomotor disturbances in addition to the foregoing complaints. On the other hand, women with pituitary failure, aside from menstrual irregularity and sterility, have few complaints. They have a tendency to obesity, although many of them lose weight, and they have lowered metabolic rates. When failure of pituitary gonadotropic hormone is responsible for the menstrual disturbance, it is not possible to detect any prolan in the urine during a period of actual failure. However, pituitary function appears to be restored sooner than ovarian function, as we have found a few cases in which small amounts of prolan were present in the urine.

We have attempted, therefore, to group these patients, by using every available means of diagnosis except the prolan test, into those we considered to have primary ovarian failure and those we considered to have secondary ovarian failure dependent on failure of the pituitary gland. It might be mentioned that estrin determinations and endometrial biopsies are included in the means of diagnosis. The reports of the prolan tests are then evaluated by this means (table 2). It is our largest group and consists of 158 women. We think it will be apparent that the prolan test verifies our diagnosis sufficiently to make us feel it is a valuable addition to the other methods of diagnosis in these cases.

TABLE 3. COMPARISON OF OVARIAN AND UTERINE RESPONSES AFTER INJECTIONS OF EXTRACTS CONTAINING PROLAN

		Number of tests showing positive ovarian responses	Number of tests showing positive ovarian and uterine[1] responses
Group I. Functional menstrual disturbances	Normal amounts	62	13
	Excess amounts	13	5
Group II. Menopause	Normal amounts	8	5
	Excess amounts	20	5
Normal women	Normal amounts	8	2
Postpartum	Normal amounts	7	0
Psychoses (menopause period)	Normal amounts	8	6
	Excess amounts	5	1
Miscellaneous	Normal amounts	2	1
Total		133	38

[1] Positive uterine response: increase in weight of 5 mg. over control.

SUMMARY

In all, 292 determinations were made on the urine of 248 women. Of these patients, 235 presented a variety of clinical conditions which we thought might be associated with abnormal gonadotropic function of the anterior lobe of the pituitary gland. Thirteen women who appeared to be normal were chosen as controls. Our largest group consisted of 158 young women who had functional menstrual disturbances. Thirty-seven women were of the menopause age, 17 were suffering from a psychosis which had developed during the menopause (a special study is being made of this group), 12 were lactating (these will also be reported separately), and 11 had some organic condition, as noted in table 3. Additional data are shown in figures 1, 2 and 3.

The clinical diagnosis in each case was made as well as could be before the prolan determination was reported. The amount of prolan found in the urine helped to substantiate the diagnosis, and in women suffering from vasomotor disturbances of the menopause the severity of the symptoms paralleled the amount of prolan per liter of urine.

REFERENCES

1. Zondek, B.: Hormone des Ovariums und des Hypophysenvorderlappens, p. 239, Springer, Vienna, 1935.
2. Katzman, P. A., and E. A. Doisy: J. Biol. Chem. **106**:125. 1934.
3. Levin, L., and H. H. Tyndale: Proc. Soc. Exper. Biol. & Med. **34**:516. 1936.
4. Frank, R. T., U. J. Salmon and R. Friedman: Proc. Soc. Exper. Biol. & Med. **32**:1666. 1935.
5. Levin, L., and H. H. Tyndale: Endocrinology **21**:619. 1937.
6. Drips, Della G.: M. Clinics of North America **21**:909. 1937.

PHOTO-COLORIMETRIC METHOD FOR THE DETERMINATION OF ANDROSTERONES IN URINE[1]

RUDOLPH NEUSTADT
From the Division of Psychiatric Research, Boston State Hospital
BOSTON, MASSACHUSETTS

Recent studies of Zimmermann (1, 2) and Oesting (3) have shown that the active R-CO-CH_2 grouping of the sex hormones reacts with m-dinitrobenzene to yield a chromogen which can be measured colorimetrically. Thus, estrone, progesterone, androsterone and pregnandione produce various colors by this reaction; the androsterone yields a violet color. Subsequently, Oesting (3) has modified the method for colorimetric measurement and reports his results as color units. Oesting (3), and Oesting and Webster (4) found a satisfactory relationship between the colorimetric assay and the comb-growth method in capons.

It is unnecessary to emphasize the advantages for clinical purposes of having an exact physicochemical method for the quantitative determination of the sex hormones instead of the more laborious and time-consuming biological methods. It is the purpose of this communication to present a physicochemical study of the color reaction of androsterone with m-dinitrobenzene and a quantitatively reliable method.

If a color solution of the androsterone extraction from urine reacting with m-dinitrobenzene is compared with a solution of 0.005N potassium permanganate (as recommended by Zimmermann) or with a blank as recommended by Oesting, considerable difficulties arise when one is working with the usual Dubosq colorimeter. First, as is well known, there are subjective differences between the colorimetric readings taken by different observers and in the repeated readings by the same individual; on the average, these differences are of a magnitude of at least 10 per cent. Second, in addition to the purple chromogen produced by the reaction of the hormone with m-dinitrobenzene, extraneous colors develop which make comparison with permanganate quite unreliable. Third, a considerable source of error is encountered because the violet pigment is transient, the color changes occurring most rapidly in the important period of from 60 to 90 minutes. Such fundamental changes in color, we felt, made the test unsatisfactory for routine clinical purposes. We, therefore, were led to determine some procedure which would eliminate the interfering colors and to elaborate a method which would determine the exact time at which readings should be taken and which would allow an exact calculation of the yielded amount at a given point of time.

[1] Aided by grants from the Commonwealth of Massachusetts and the Rockefeller Foundation; personal grant from the Emergency Committee in Aid of Displaced Foreign Physicians.

METHOD

The extraction method is essentially similar to that described by Oesting. A 24-hour specimen is obtained and measured; 1000 cc. of the 24-hour specimen are brought to a pH of below 1.0 with concentrated sulfuric acid and used for further examination. For a practical assay, the amount of 1000 cc. was found to be the proper one. The acidified urine is autoclaved for 15 minutes under a pressure of 15 lb., cooled, and then extracted with benzene at least 12 times. It is necessay that in every instance only the purest benzene be used. Since we used a different extraction apparatus from that employed by Gallagher, Koch and their co-workers (5), it was impossible for us to finish the 'twelve times' extraction in 15 minutes as they did, but rather in about 60 minutes. We found that, on the average, the amount of androsterone is 30 per cent to 40 per cent higher with the shorter extraction period of 12 times than with the longer period of from 16 to 24 hours, such as was used in the beginning of our work. However, this difference between the short method and the long extraction was not found in a few cases having a low amount of androsterone.

After distilling off the benzene, the completely cooled urine is transferred to a separatory funnel. It proved to be very important that the distillation flask be washed with at least 100 cc. of ether, as well as with 50 cc. of 10 per cent aqueous NaOH solution. The amount of androsterone yielded was further increased 10 per cent to 20 per cent by washing out the distillation flask before shaking the 3 substances in the separatory funnel. For this reason, our results are somewhat higher than those of some of the former investigators. Furthermore, we found that 100 cc. of ether is not enough to dissolve the residue of 1000 cc. urine, so we use about 250 cc. The ether solution in the separatory funnel is washed 2 or 3 times with 50 cc. portions of 10 per cent aqueous solution of NaOH and 2 or 3 times with 25 cc. portions of 10 per cent aqueous solution of NaOH. In the next stage we again followed Oesting in washing the ether solution 3 times with 30 cc. portions of distilled water, transferring it to a beaker and stirring with 0.5 gm. decolorizing charcoal. This is filtered through a Whatman No. 40 dry filter paper into a distillation flask. The remaining charcoal and the separatory funnel are washed with about 70 cc. of ethyl ether and the filtrate thereof combined with the first filtrate. Then the ether is distilled off on a hot water bath to dryness, and the distillation flask is stoppered with a calcium chloride tube during the last minutes of the distillation to prevent any traces of water entering the flask.

After slowly cooling to room temperature, the residue in the distillation flask is carefully dissolved in 20 cc. of 60 per cent alcohol. The 20 cc. of the alcohol-hormone solution is divided into 2 equal parts of 10 cc. each and transferred to a special tube for colorimetric purposes. It is important to measure the 10 cc. accurately, since even a small variation influences the reading and calculation. To each test-tube are added 2 cc. of a 2 per cent alcoholic solution of m-dinitrobenzene (2 gm. in 100 cc. of 95 per cent alcohol) and 2 cc. of a 15 per cent aqueous solution of KOH. The ratio 10:2:2 has to be observed carefully, because any change in this ratio influences fundamentally the time in which the chromogen is formed, as has already been shown in Zimmermann's (1, 2) studies. This fact is to be kept in mind in those cases in which the chromogen is so dark that a dilution for the purpose of a more exact calculation is desirable. In those cases the content of the test-tube is to be diluted with 14 cc. of a solution which is composed of 10 cc. of 60 per cent alcohol, 2 cc. of 2 per cent m-dinitrobenzene and 2 cc. of 15 per cent KOH. There are a few more necessary precautions. First, the ether solution should

be absolutely free from color after its filtration; second, there should be no traces of charcoal in the distillation flask; and third, the m-dinitrobenzene should be free from all additional color-giving substances. It may be necessary to recrystallize the m-dinitrobenzene in 80 per cent alcohol once or several times.

The content of the test-tubes is mixed, and the tubes are placed in the dark. Readings are made after exactly 30, 60 and 90 minutes respectively. Although the calculation is derived from the reading after 90 minutes, it is also worth while to take the readings after 30 and 60 minutes. The readings for both test-tubes should be identical. However, differences of 0.25 or 0.50 may occur. A difference greater than 0.75 usually signifies that an error has been made. This is mostly due to the presence of a trace of charcoal in one of the test-tubes. Another filtration before the final reading will remove it and help toward coincidental readings.

The colorimetric readings are taken in an Evelyn photo-electric colorimeter (6) or by the use of the spectrophotometer. If the Evelyn colorimeter is used, a Corning glass filter is selected, which transmits a narrow spectral band in the vicinity of 500 to 520 mμ. A blank of 60 per cent alcohol, placed in an Evelyn tube and transferred to the Evelyn colorimeter, using a Corning filter transmitting light at 500 to 520 mμ, yields a center setting of the galvanometer string without the reagent at 68. Having adjusted the colorimeter exactly to this point in advance, one can easily make 6 to 8 readings during one minute, and any subjective error is excluded. The calculation follows the formula: $\frac{2 - \log G}{K_2}$, as shown later. Thus, the examination is completed in 5 to 6 hours.

There is still one point concerning the method to be explained, i.e., the amount of 1000 cc. urine selected for extraction. Working with both hospitalized and non-hospitalized patients, we preferred to make the extraction and calculation on the basis of 1000 cc. of urine as a routine. We found that, with the hospitalized patients, from whom we were sure the whole 24-hour specimen was obtained, the calculation on the basis of the whole daily excretion was more accurate and constant than the calculation per 1000 cc., but with the non-hospitalized patients, where in some cases we could not be sure of the entire daily excretion, the calculation for 1000 cc. seemed to be more exact. The calculation for daily excretion can easily be made in addition to that for 1000 cc. of urine.

EXPERIMENTAL

A). Selection of the filter. The spectrophotometric curve of the purple chromogen produced by the reaction of androsterone with m-dinitrobenzene is a single absorption band at 510 mμ as determined by spectrophotometric measurement.[2] The final chromogen derived from 2 mg. and 4 mg. of androsterone give optical densities in the spectrophotometer of 0.18 and 0.36 at 510 mμ. Thus, the color relationship appears to be stoichiometric.

The Corning glass filter No. 520 of the Evelyn apparatus transmits the light at the vicinity of 510 mμ wave length, thus corresponding to the chromogen derived from androsterone (fig. 1).

Under normal conditions, only the filter No. 520 is to be used for the readings of the androsterone chromogen. It was found that the colorimetric reactions of trans-dehydro-androsterone were almost identical with those of androsterone. Only in special cases, for instance, after treatment with tes-

[2] Spectrophotographs were made by the Massachusetts Institute of Technology.

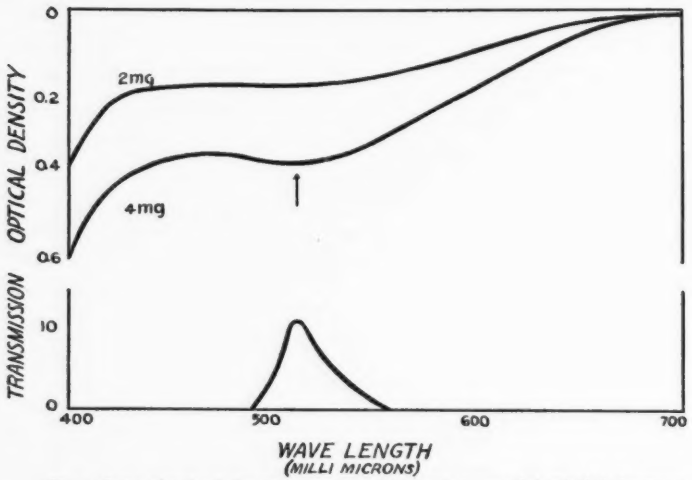


Fig. 1. Curve, showing in its upper part the stoichiometric nature of the yielded chromogen and optical density at 510 mμ; in its lower part the light transmission of the filter with its corresponding peak at 510 mμ.

tosterone derivatives, a second (brown) color may develop which requires readings with two filters, No. 420 and No. 520. This necessitates a more complicated calculation for both chromogens. We shall deal with this procedure in a later publication.

B). The time-factor. As already mentioned, our calculation is derived from the reading after 90 minutes. The reading at this point was found to be most reliable, as shown in table 1 and figure 2.

Figure 2 graphically demonstrates the results in table 1.

These curves show clearly that the reaction is not yet fully completed after 30 minutes, the 30-minute curve having a course different from all others.

TABLE 1. GALVANOMETER READINGS

Minutes	1 mg.		2 mg.		3 mg.		4 mg.		6 mg.		8 mg.	
		%		%		%		%		%		%
30	85	40	70	43	62	39	55	32	41	37	32	36
60	76	67	53	78	42	71	33	68	20	66	12	65
90	70	*89*	43	*104*	30	*100*	19	*100*	9	*99*	4	*99*
120	63	114	33	137	22	124	15	117	8	104	3	104
150	60	127	28	157	18	141	12	131	7	109	3	108
180	56	143	24	176	15	156	9	158	6	116	2	121

Such an irregularity was often found after 30 minutes, and in a few specimens it was still present after 60 minutes. On the other hand, figure 2 shows that the process is almost completed within 2 hours. Calculation after this time would be difficult, because all of the readings are too close together to give an exact computation. The curves having the most ideal structure are found after 60 and 90 minutes. We chose the 90-minute curve for the final reading and calculation in order to exclude any irregularity of the velocity

of the reaction at its beginning. At the same time, the 90-minute reading has the advantage also of being near to the maximum peak of the reaction. According to the stoichiometric nature of the reaction the 90 minute readings with from 1 to 8 mg. of androsterone[3] give an ideal parabolic curve.

Thus, we derived the K_2 factor from the reading after 90 minutes. Table 1 shows in each second column the percentage of androsterone calculated with the K_2 factor of 0.176, the derivation of which is given below. Table 1 shows in this column that only with very small amounts of androsterone (1 mg. or less) is there a deviation from the mathematical expectation. In the range from 2 to 8 mg. (the norm in human urine ranging from 4 to 7 mg. per liter)

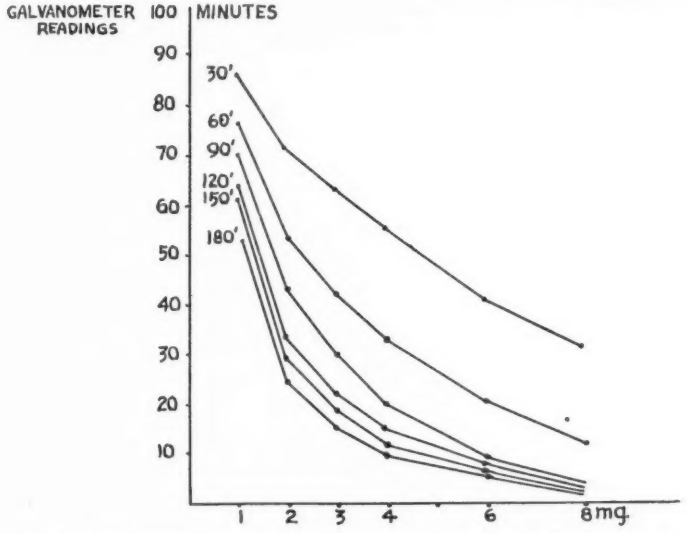


Fig. 2. Graphic demonstration of the corrected galvanometer readings of from 1 to 8 mg. androsterone in from 30 to 180 minutes.

the coincidence between the used amount of androsterone and the 90-minute readings is perfect; the mean error not exceeding $\pm$ 0.02.

C). Calculation. Since the color reaction is stoichiometric, the equation based upon the law of Lambert and Beer can be applied:

1) $C = \frac{L}{K_1}$.

C is the concentration of androsterone per cc. of final volume; $L = 2 -$ log G (galvanometer reading) or more correctly $2 -$ log G_1 (corrected galvanometer reading according to the individual deviation of each apparatus); and K_1 is a factor for the relationship of the filter used and the chromogen present. Thus, if we use a solution of 4 mg. of androsterone in 28 cc. of solution, the amount of androsterone present is 0.142 mg. per cc. We have found by experiment that $L = 0.704$, if the reading is taken after 90 minutes.

[3] We are indebted to Dr. E. Oppenheimer of the Ciba Pharmaceutical Products for providing us with the necessary amount of androsterone and dehydroandrosterone.

Thus,

$$0.142 = \frac{0.704}{K_1} \text{ or}$$

$$2)\ K_1 = \frac{0.704}{0.142} = 4.95.$$

A new constant can thus be derived according to the general equation:

$$3)\ K_2 = \frac{A}{V} \times K_1,$$

where A = aliquot and V = final volume. In our system $A = 1$ (liter) or, as derived from 1000 cc. of urine, $A = \frac{1000}{1000}$; $V = 28$. Thus,

$$4)\ K_2 = \frac{4.95}{28} \times \frac{1000}{1000} = 0.176.$$

Thus, the final formula is:

$$5)\ \frac{2 - \log G}{0.176} \times \frac{1000}{1000} = \text{mg. androsterone per liter of urine.}$$

The result can be expressed in miligrams, international units (1 mg. = 10 I.U.) or gamma per liter. By introducing the value for the amount of urine received, expressed in cubic centimeters, into the nominator of our formula, the amount of androsterone per daily excretion is determined. Introducing the number of cubic centimeters in the denominator of our formula, in case an amount other than 1000 cc. is used for extraction, gives the amount of androsterone per liter of urine at once.

CONCLUSIONS

The method has proved to be reliable and adapted to clinical purposes. We have already performed 125 examinations in 3 months, the results of which were therapeutic and diagnostic *guides* in many instances.

We have tested the value of the method by adding a known amount of crystallized androsterone to one-half of a specimen of urine, the other half of which was examined the same day. With improvement of the technic and longer experience, the results also improved, as shown in table 2. This is another proof of the value of the method for practical purposes.

TABLE 2

Examination Number	I.U. per Liter	Added Amount of Androsterone in mg.	I.U. in Second Specimen	% Amount Recovered
26/27	66.7	2.7	81.5	55
29/30	16	3.2	56	120
31/32	32.8	2.7	52.8	74
33/34	53.6	2.7	80.8	100
35/36	40.5	2.7	67.6	100
39/40	29.8	2.1	49.8	98
41/42	45.1	2.0	64.7	97

It may be well at this time to add the fact that the results are not influenced by leaving the gathered urine for 24 hours or longer in the ice-box.

The advantages of precise color measurements of this character follow. *a)*, It reduces the outlay of work; *b)*, The subjective error is practically excluded; *c)*, The use of a suitable filter excludes interfering colors; *d)*, The examination is easily and quickly performed; *e)*, It gives an exact calculation, which is necessary for clinical purposes.

SUMMARY

A photo-colorimetric method is described for the determination of androsterones in urine. The method is rapidly performed and yields an exact calculation of the excreted amount of androsterones in milligrams, international units or gamma per liter.

I hereby express my gratitude to Dr. Abraham Myerson, director of this laboratory, whose interest in the problem of hormones in the psychoses and neuroses led to my study of this subject; to Dr. Michel Pijoan of Harvard Medical School for his criticism of my work; and to Miss L. Gladys Howard for her untiring help.

REFERENCES

1. Zimmermann, W.: Ztschr. f. Physiol. Chem. **233**:257. 1933.
2. Zimmermann, W.: Ztschr. f. Physiol. Chem. **245**:47. 1936.
3. Oesting, R. B.: Proc. Soc. Exper. Biol. & Med. **36**:524. 1937.
4. Oesting, R. B., and B. Webster: Endocrinology **22**:307. 1938.
5. Gallagher, T. F., D. H. Peterson, R. J. Dorfmann, A. T. Kenyon and F. C. Koch: J. Clin. Investigation **16**:695. 1937.
6. Evelyn, K. A.: J. Biol. Chem. **115**:63. 1936.

THE SPECIFIC METABOLIC PRINCIPLE OF THE PITUITARY, AND ITS RELATION TO THE MELANOPHORE HORMONE

D. K. O'DONOVAN[1] AND J. B. COLLIP
From the Department of Biochemistry, McGill University
MONTREAL, CANADA

In two preliminary communications from this department (1, 2) the presence of a metabolic stimulant in pituitary extracts, other than the thyrotropic hormone, has been reported. This principle causes a sharp rise in oxygen consumption within a few hours after injection, with a corresponding fall in the respiratory quotient. Its probable identity with the melanophore hormone of the pars intermedia has been suggested. The present paper contains a more detailed account of the work on this subject.

Previous workers have shown that pituitary extracts may stimulate metabolism even in thyroidectomized animals. Houssay and Artundo (3) found that in thyroidectomized dogs the metabolic response to pituitary extracts was reduced but still present. Gaebler (4), using an impure growth preparation in a single massive dose subcutaneously, showed a well-marked rise in oxygen consumption of thyroidectomized dogs, which was at a maximum after 24 hours and persisted for some days. The respiratory quotient remained low. The effect was abolished by boiling for five minutes (5). Riddle, et al. (6) found that a prolactin-growth preparation stimulated metabolism in both normal and thyroidectomized pigeons.

Fisher, Russell and Cori (7) confirmed the observation of Fisher and Pencharz (8) that fasted hypophysectomized rats have a higher R.Q. than normal controls. They found that an anterior pituitary extract depressed the R.Q. to normal levels, .767 and .726 respectively. From a detailed study of carbohydrate concentrations, and apparently because the oxygen consumption was depressed after injection, they attributed to the pituitary gland an inhibitory action on carbohydrate oxidation. Meyer, Wade and Cori (9) and Russell (10) performed similar experiments on normal glucose-fed rats. These authors found an increase in oxygen consumption with lowering of the R.Q.; nevertheless, they interpreted their results as a simple depression of carbohydrate metabolism. The activity of the extract was in each case destroyed by boiling for less than half an hour.

The only evidence available of an immediate stimulation of metabolism with pituitary extracts comes from workers with thyrotropic preparations. Krogh and Okkels (11) and Diefenbach (12), using rabbits, guinea-pigs and rats, reported a rise in the basal metabolic rate within two hours after subcutaneous injection. Zunz and LaBarre (13), by indirect means, also postulated an immediate action of the thyrotropic hormone on dogs.

PROCEDURE

Rabbits of a Chinchilla breed, weighing 2 to 2.6 kg., and obtained from a single source, were used throughout the experiments. This particular breed

[1] Assisted by a grant from the Irish Medical Research Council.

was chosen for its relatively low and constant B.M.R. Both sexes were used, but females were more adaptable to training and gave more consistent results. The diet consisted of ground oats and clover hay, with fresh vegetables once a week. The animals were kept at least one month in the laboratory before inclusion in an experiment, preferably in single cages. During this time they were accustomed to the requisite manipulations for the estimation of oxygen consumption. The latter was estimated by a modified open Haldane technic in a constant-temperature room over periods of at least one hour. When the R.Q. was observed, the period was extended to two hours. The apparatus was checked by combustion of ethyl alcohol under conditions, as regards time and rate of oxygen consumption, which were almost identical with the actual experiments: average of 10 = .675, or .008 above the theoretical value (max. = .680; min. = .664).

In the initial experiments the animals were fasted 24 hours, but to facilitate frequent estimations on each rabbit, this point was disregarded, and the only precaution taken was to avoid a large food intake during the previous 12 hours. The question of fasting will be discussed more fully below.

Animals were discarded if they showed restlessness in the jar, or if controls showed a wide variation. Similarly, individual results were discarded where the animal was not at rest during an experiment. It is worthy of mention that the pituitary extracts used rarely, if ever, produced restlessness, although respiration may be stimulated both in rate and depth during the period of increased metabolism. With large doses a marked hyperpnea was sometimes observed.

The procedure adopted to study the immediate effects of pituitary extracts on metabolism was as follows. After a control estimation of the resting oxygen consumption (referred to as the pre-injection level) the extract was injected and a varying number of estimations were taken during the following 4 to 8 hours. No food was given during this period. The data are expressed here as liters of oxygen per square meter of body surface per 24 hours (Meeh's formula, and Rubner's $K = 12.5$). The midpoint of each estimation is recorded as the time after injection (see fig. 1 for typical results). In order to condense the results, the highest point on the post-injection curve is expressed as a percentage change from the pre-injection level. Different extracts may have a varying rate of absorption, for example, a simple alkaline extract (14) from sheep anterior pituitary may not give the peak-point of metabolism until the seventh hour after injection, whereas a similar extract from ox and the more highly purified extracts described below usually gave the maximum effect at the third to the fourth hour. The increased oxygen consumption may be maintained over the subsequent 10 hours; in only 3 instances was there an appreciable effect over 24 hours.

To study the variation in metabolism from hour to hour, as well as the disturbing influence due to injection, 10 rabbits each received 4 cc. of water (the same volume as that of extract used) subcutaneously after an initial record of oxygen consumption. During the following 5 hours, a total of 34 records of metabolism were obtained and gave the following analyses when expressed

as percentage change from the pre-injection level: average = 103.7 per cent; max., +12 per cent; min., —8 per cent; standard deviation = ±4.7. Further, the effects of crude alkaline extracts made from kidney, liver and muscle, were studied in 4 rabbits not used in the above group. Each animal received 5 cc. of each extract (equivalent to 1 gm. tissue) intraperitoneally on 3 consecutive days. Analyses of 46 post-injection results showed an average of 99.6 per cent (standard deviation = ±7.1). In no case was there a typical curve as found with pituitary extracts.

Most of the work concerning assays and the study of the chemical and physical properties of the active principle was done on chronically injected animals. The injections were spaced at approximately two per week to allow a sufficient recovery period. This method has the advantage of using an animal of known sensitivity. If, for any reason, usually abscesses or middle ear disease, an animal became refractory, it was discarded. A detailed study of resistance is not included in this communication.

The melanophore hormone activity of extracts was assayed on frogs. The total volume injected into the dorsal lymph sac was 0.5 cc. The required dilutions were made with saline, and the pH was on the alkaline side of neutrality. Saline controls were included in each group. Blood sugars were estimated by the method of Hagedorn and Jensen (15). The CO_2 combining power of plasma was estimated by the method of Van Slyke and Cullen (16), and blood urea concentration by the method of Van Slyke and Cullen (17).

TABLE 1. NORMAL RABBITS: 4 CC. PITUITARY EXTRACT *A*

	Serial No.	Sex	Aver. of controls[1]	Pre-inj. level[1]	Post-inj. max. change %
Intraperitoneal	11	F	127	122	+38
Intraperitoneal	12	M	143	151	+25
Intraperitoneal	13	M	143	137	+17
Intraperitoneal	14	M	131	134	+20
Intraperitoneal	15	F	119	122	+42
Subcutaneous	18	M	141	141	+21
Subcutaneous	19	M	144	141	+28
Subcutaneous	16	F	129	125	+35
Subcutaneous	21	F	138	129	+47
				Average	+30

[1] Liters of oxygen per sq. m. of body surface per day.

EXPERIMENTAL RESULTS

Table 1 shows the immediate effect of pituitary extract *A* on oxygen consumption. This extract is a powerful thyrotropic preparation and was made from dissected anterior lobes of ox pituitaries by alcohol precipitation of a concentrated aqueous extract made at pH 5 from glands that had been extracted with acetone and subsequently treated with aqueous alkali (pH 10) for 4 hours. Evidence that this immediate effect is distinct from the thyrotropic hormone is given in table 2, where the same extract was given to 9 thyroidectomized rabbits within 8 days after operation. The response was less in the latter group, but the difference is not considered significant, owing to wide

TABLE 2. EFFECT OF THE THERMOSTABLE METABOLIC STIMULANT ON THYROIDECTOMIZED RABBITS

	Serial No. and sex	% Change with inj. within 1 wk.	% Change after 3–6 wk.	Residual thyroid mg.	% Change in B.M.R. due to thyroidectomy; 3–6 wk.
4 cc. Ext *A*, boiled[1] 1 hr., subcutaneously	22M	+34		None	
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	35M	+29		8	
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	47M	+11		5	
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	45F	+17		24	
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	36F	+ 8	+12	None	−44
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	38F	+ 9	+25	None	−32
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	39F	+18	+12	15	−28
4 cc. Ext. *A*, boiled[1] 1 hr., subcutaneously	31F	+21	+55	18	−33
4 cc. *A.I.F.* subcutaneously	43M[2]	+26		None	
	Average	+19	+26		

[1] Here and elsewhere 'boiled' refers to heating submerged in a boiling-water bath.
[2] *R 43* received injections before operation. The day before it showed +28%, the day after +25% and 2 days after +26%.

individual variation; also, *R 31* had been tested one month before operation and had shown a similar response, and *R 26* had been injected for more than one month before thyroidectomy. Removal of the thyroid did not alter the response on the first or second day after operation. Table 2 also shows a similar effect in some of the same animals after an interval sufficient to produce some thyroid insufficiency, as indicated by the B.M.R. The response in the latter group differed since the peak occurred nearer the sixth hour rather than the third, as in normal or recently thyroidectomized animals. It should be noted that the rise is relatively less than the actual value of +26 per cent would indicate, since it is calculated on a much lower pre-injection level.

Further evidence that the immediate rise is due to a factor other than the thyrotropic hormone is obtained from the relative resistance of the two sub-

TABLE 3. INFLUENCE OF HEAT ON METABOLIC STIMULATION

4 cc. Pituitary Extract *A*	Rabbit serial no.	Sex	Pre-injection level[1]	Post-injection change %
Boiled 2½ hr.	23	F	103	+32
Boiled 2½ hr.	24	M	134	+19
Boiled 2½ hr.	25	M	128	+19
Boiled 1 hr.	26	F	125	+24
Boiled 1 hr.	26	F	128	+22
Boiled 1 hr.[2]	19	M	127	+25
Boiled 1 hr.[2]	16	F	114	+39
Boiled 1 hr.[2]	21	F	107	+49
			Average	+28.7

[1] Liters of oxygen per sq. m. of body surface per day.
[2] *R19, 16, 21* were 3 chronically treated animals. Controls with unboiled pituitary extract had rates respectively, of +47%, +42%, and +14%.

TABLE 4. EFFECT OF A SINGLE DAILY INJECTION OF 4 CC. THYROTROPIC EXTRACT *A* (BOILED FOR 1 HR.) ON OXYGEN CONSUMPTION. RABBIT *29*, FEMALE

No. days injected	1	2	3	4	10	17	24	31	38	45	49	63	72
Pre-injection level	122	140	140	137	136	125	120	129	131	130	117	112	106
Post-injection maximum	169	173	178	159	158	157	168	174	157	156	165	159	153
Per cent increase	39	24	27	16	16	26	40	35	20	20	41	42	44

Average of 5 controls (before treatment) = 131 (max. 137; min. 122).

stances to high temperature. The thyrotropic hormone is destroyed in a short time by the temperature of the boiling-water bath (18). Recent work from this department (19) requires the above statement to be qualified by stating the pH of the extract. This pituitary extract *A*, when boiled for one hour at approximately pH 7, contains no appreciable amount of thyrotropic hormone, as indicated by its failure to produce hyperplasia in the thyroid of treated guinea pigs. Table 3 shows that it was still active, in so far as the immediate rise in metabolism is concerned, even after boiling for 2.5 hours.

Animals injected daily for a long period may show at first a diminishing response compared with the first day. Later the pre-injection level of metabolism tends to fall below the level of controls, but with a typical high daily rise after injection. This is exemplified by data shown in table 4 where the daily fluctuations in oxygen consumption are recorded. The extract was boiled for one hour to destroy any thyrotropic activity, so that the fall in the pre-injection level is not due to the formation of the antithyrotropic substance. No resistance was developed to the immediate rise even after 112 days of injection under the conditions of this particular experiment. The data recorded in table 4 illustrate typically the findings on a series of 10 animals injected over a long period of time, but not so completely studied. Nevertheless, the possibility of resistance must be borne in mind since an animal may become refractory to frequent daily injections. Table 5 shows the result of increasing the number of daily injections. The animal used had been injected once daily over a prolonged period and the daily response was approximately +40 per cent. As the number of daily injections increased, the response rapidly diminished. A

TABLE 5. EFFECT OF FREQUENT INJECTIONS (3 PER DAY) OF PITUITARY EXTRACT *A* ON OXYGEN CONSUMPTION TAKEN BEFORE AND 3 HOURS AFTER INJECTION[1]

Date, October	25	26	27	28	29
1st inj.	118–172	116–166	138–173	117–145	131–143
2nd inj.	No inj.	—	137–164	125–140	128–142
3rd inj.	No inj.	—	—	—	—

[1] Rabbit *19*. Male. Chronically injected for 2 months with a single daily injection. The normal oxygen consumption before treatment was 144 (average of 5); max. 155, min. 134.

similar refractory state was developed in an animal, previously responsive, by fasting for 4 days, with continuation of a single daily injection.

The substance responsible for the immediate metabolic response is probably present to a greater or lesser degree in almost any pituitary extract. Thyrotropic or gonadotropic preparations, which as a rule represent aqueous extracts

TABLE 6. INFLUENCE OF STEHLE'S PURIFIED MELANOPHORE HORMONE ON METABOLISM

Dose, mg.	Sex	Serial No.	Pre-injection Level	% Change B.M.R.
2	M	19	130	+40
2	F	21	128	+16
2	F	16	133	+14
2	F	21	114	+18
2	F	28	126	+20
2	F	29	124	+28
0.5	M	42	115	+15
2.5	F	52	92	+61
			Average	+26.5

that have been precipitated by 70 per cent alcohol, contain the active substance. One of the best preparations which we have was made by concentrating to an aqueous phase the alcoholic filtrate obtained by precipitating the thyrotropic and gonadotropic substances. This solution, after ether extraction, was saturated with ammonium sulphate and the precipitate removed. This was taken up in water and again precipitated with ammonium sulphate. The aqueous solution of the precipitate was made up to 90 per cent alcohol with absolute alcohol and the resultant precipitate removed, dissolved in water and again precipitated by 90 per cent alcohol. The aqueous solution of this precipitate was precipitated isoelectrically and the filtrate was found to be rich in the metabolic factor. It contained 1.13 per cent total solids, including 0.5 per cent $(NH_4)_2\ SO_4$, and is referred to hereafter as *'A.I.F.'* It was a powerful metabolic stimulant, but had only slight activity when assayed for prolactin and adrenotropic hormone. The high melanophore hormone content of this extract (0.5 cc. of 1/5000 dil. = 1 frog unit) as well as of the thyrotropic fractions, suggested that this hormone may be identical with the metabolic stimulant.

Using some of Professor Stehle's (20) preparation of melanophore substance made from posterior lobe powder, of which $1\gamma = 1$ frog unit, a dose of 2 mg. was sufficient to cause a well-marked rise in metabolism (see table 6); 0.5 mg. to one animal also caused a significant increase.

On the other hand oxytocin (postlobin-O) (21) produced no change in metabolism of 3 rabbits of known sensitivity in doses of 15 to 30 U. Similar doses of the posterior pituitary pressor principle (postlobin-V) given to the

TABLE 7. INFLUENCE OF BOILING AT PH 10 FOR 10 MINUTES ON THE IMMEDIATE RISE IN CHRONICALLY INJECTED RABBITS

Pit. Ext.	Dose cc.	Sex	Serial No.	% Change B.M.R. (unboiled)	% Change B.M.R. (boiled pH10)
M	4	M	42	+31	+30
M	4	M	49	+20	+23
A.I.F.	4	M	42	+40	+46
A.I.F.	4	M	49	+27	+27
Stehle's Melanophore Powder	3 mg.	F	52	Boiled in N/10 NaOH for 15 min.	+41

same animals produced in two cases a marked fall in metabolism during the first hour, followed by a slight compensatory rise at about the second or third hour (max. +12 per cent). This is in agreement with the results of Geiling and DeLawder (22) and Grollman and Geiling (23). The rise in metabolism produced by the melanophore extract is not to be confused with the slight compensatory rise produced by the pressor principle. The melanophore extract of Stehle and other extracts producing the immediate rise showed no preliminary fall. Figure 1 shows typical examples of the action of all three extracts.

Since the melanophore hormone is very resistant to alkali, and is possibly potentiated by boiling in N/10 NaOH (24, 25), we have studied the effect

TABLE 8. INFLUENCE OF EXTRACTS OF PARS INTERMEDIA AND PITUITARY COLLOID (OX)

Serial no. and sex	Extract	Dose cc.	Hours after injection	% Change in B.M.R.	R.Q. Before	R.Q. After
42M	Colloid (4)	0.5	2–4	+18	—	—
48M	Colloid (4)	0.4	2–4	+23	—	—
49M	Colloid (4)	0.4	2–4	+14	—	—
51F	Colloid (4)	1.0	2–4	+75	.86	.76
			6–8	+41		.74
51F	Colloid (32)	1.0	2–4	+ 4	.90	.90
51F	Colloid (32)	1.0	2–4	+12	.79	.76
51F	Pars Intermedia	1.0	2–4	+21	.91	.85
			6–8	+ 2		.85
51F	Colloid (4)	1.0	2–4	+19	.89	.81
			6–8	− 9		.90
53F	Pars Intermedia	1.2	2–4	+30	.84	.77
			6–8	+14		.79
54M	Pars Intermedia	0.5	2–4	+ 3	.75	.74

of boiling some pituitary fractions at pH 10 for 10 to 15 minutes. The data are summarized in table 7. There was no evidence of a quantitative change in the response. It should be noted that these extracts had all been subjected to dilute alkali at room temperature in the initial stage of preparation.

Specimens of pituitary colloid and an extract of dissected pars intermedia obtained from Dr. E. B. Astwood[2] (26) were assayed for their content of the melanophore hormone and their influence on metabolism. Pituitary colloid is generally conceded to contain the chromatophore hormone(s) in high concentration (27, 28). The pars intermedia is recognized as the source of the melanophore hormone in most mammals. Doses of 0.4 cc. of the colloid extracts, equivalent to 9 mg. of original colloid, produced a typical rise in metabolism. Extracts of the pars intermedia had a similar potency (table 8).

Table 9 gives a summary of the various pituitary extracts, their source, method of extraction, melanophore hormone content, and effective metabolic dose. Owing to wide individual variation in animal sensitivity, a constant ratio cannot be expected. Nevertheless, the results indicate a relationship between the two substances.

A single injection of an extract made from 200 mg. of anterior pituitary powder of the finback whale by boiling in N/10 NaOH for 10 minutes pro-

[2] Johns Hopkins Hospital, Baltimore, Md.

duced a significant rise in oxygen consumption (+19 per cent). Valsö (29) and Geiling (30) have shown that the anterior lobe of this species contains the melanophore hormone, whilst the anatomically separate posterior lobe contains both oxytocin and vasopressin.

The metabolic principle is easily soluble in water and insoluble in acetone. It usually retains its activity on storage at low temperature in 10 per cent alcohol even as long as two years. But the potency of some extracts rapidly diminished during storage. Thus an extract (*Poly-B*) made 8 months previously had lost most of its activity on metabolism and on frog melanophore (see table 19). This was generally noted in the case of primary unfractionated

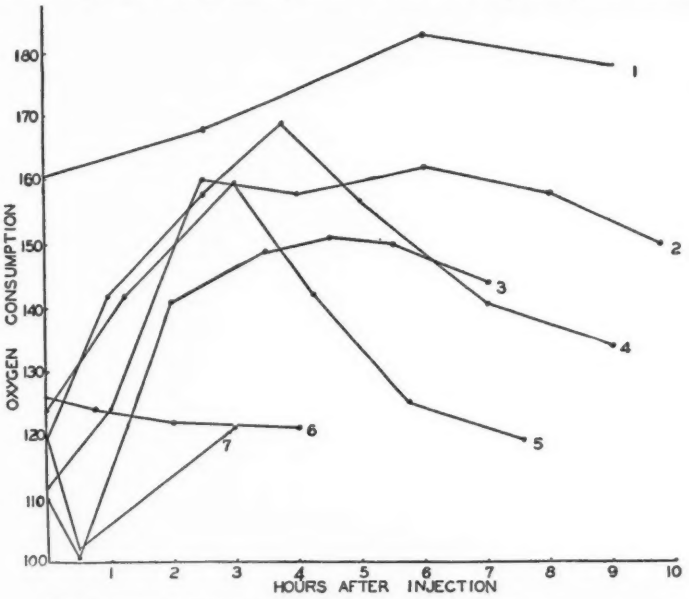


Fig. 1. Examples of metabolic changes with various pituitary extracts.
Curve 1. Rabbit 9. Crude alkaline extract of sheep anterior pituitary; 5 cc. intraperitoneally = 1 gm. of gland. *Curve 2.* Rabbit 30. Crude alkaline extract of ox anterior pituitary; 5 cc. intraperitoneally = 1 gm. of gland. *Curve 3.* Rabbit 16. 4 cc. *A.I.F.* subcutaneously *Curve 4.* Rabbit 29. Extract *A;* thyrotropic fraction, boiled at pH 7 for 1 hour; 4 cc. subcutaneously. *Curve 5.* Rabbit 29. 2 mg. Dr. Stehle's posterior pituitary melanophore powder subcutaneously. *Curve 6.* Rabbit 29. Postlobin-O (oxytocin); 15 U subcutaneously. *Curve 7.* Rabbit 29. Postlobin-V (vasopressin); 15 U subcutaneously.

and unboiled aqueous extracts. The possibility of the loss in potency on storage being due to enzymatic destruction of the active principle is being investigated by our colleague Dr. O. F. Denstedt. The active principle is stable in dilute acids (pH 5). Its resistance to heat and alkali has already been noted. It is adsorbed by charcoal, if the charcoal is slowly filtered off (table 10). However, if it is centrifuged off after 2 minutes at room temperature, the supernatant may contain an appreciable amount of melanophore and metabolic activity. Jores (31) and Dietel (32) have published different views on adsorption of the melanophore hormone. The former held it as an essential characteristic, while the latter claimed it was poorly adsorbed. We find both activities are

susceptible to prolonged tryptic digestion (48 hours at pH 8.5) (table 11), but in some earlier experiments, after 12 hours of digestion, there was a negligible loss. These results are not in agreement with Hogben and Winton (33), but a low efficiency of the trypsin may have influenced our results. Both principles are resistant to pepsin at pH 3 for one-half hour (fig. 2).

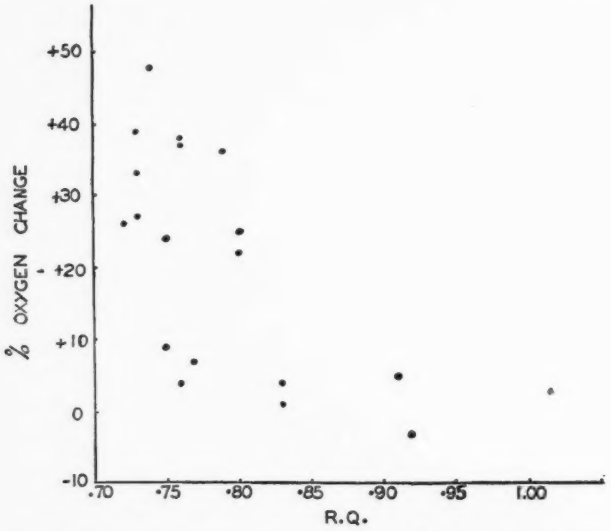


Fig. 2. The relation of oxygen consumption to R.Q. at the 6th to 8th hour after injection of 4 cc. of extract *A.I.F.* subcutaneously. The animals were fasted 2 to 48 hours. The data include two which were obtained with peptic digest of the extract (+22 and +26%).

TABLE 9. DETAILS OF PITUITARY EXTRACTS CONTAINING THE SPECIFIC METABOLIC HORMONE

Extracts and Source	Thyrotropic *A* from ant. lobe of ox	*A.I.F.* from whole gland of sheep	Stehle's melanophore from post. pit. powder	Pit. colloid from ox	Extract of pars intermedia of ox	*Poly-B* pig ant. lobe
Method of Extraction	70% alcohol ppt.	90% alcohol ppt.	Dilute acetic acid extr. Filtrate from abs. alcohol ppt.	0.25% acetic acid extr.	0.25% acetic acid extr.	Aqueous extr. at pH 5
Minimal Frog Melanophore Unit	0.5 cc. of 1/2500 =3.6γ	0.5 cc. of 1/5000 =0.67 γ	1 γ	0.5 cc. of 1/50,000	0.5 cc. of 1/20,000	0.5 cc. of 1/15,000 =0.17 γ
Dose Producing Metabolic Increase	4 cc.=72 mg. 2 cc. active	4 cc.=25 mg. (prolonged effect) 1 cc. active	2 mg.	1 cc. 0.4 cc. active	1 cc.	1 cc.=5 mg. prolonged effect
Equivalent	2 cc.=3 gm. ant. lobe	1 cc.=4 gm. whole pit.	2 mg.=500 mg. post. pit. powder	0.4 cc.=9 mg. colloid	—	1 cc.= 0.41 gm.

TABLE 10. INFLUENCE OF CHARCOAL ADSORPTION ON EXTRACT *A.I.F.*[1]

	Sex	Dose	Hours after Injection	% Change B.M.R.	R.Q. Before	R.Q. After
R52	F	1 cc. Filtrate	2–4	+2	.75	.78
R52	F	3 cc. Filtrate	2–4	+ 8	.91	.88
			6–8	+ 7		.81
R52	F	Control[2]				
		3 cc. *A.I.F.*	2–4	+34	.80	.92
			6–8	+39		.73

[1] Frog unit less than 0.5 cc. of 1/100 dilution.
[2] Marked hyperpnea during the first 3 hours after injection.

Rabbits having a high metabolic rate after injection with pituitary extracts show no evidence of increased muscle tonus; on the contrary, muscular relaxation is more characteristic. The nature of the response, particularly its transitory character, might suggest a secretion of adrenalin. Alteration in blood sugar is considered to be the most sensitive indication of adrenalin secretion in rabbits. Table 12 shows blood sugar response of a group of rabbits after injection with various pituitary extracts in doses sufficient to cause more than 20 per cent increase in B.M.R. at the third hour. The curves show a slight drop in blood sugar coinciding with the increased oxygen consumption, whilst control subcutaneous injections of adrenalin in amounts insufficient to increase metabolism caused a marked hyperglycemia.

For the study of blood sugars, extracts made from the dissected anterior lobe were designedly used, owing to the variable influence of posterior pituitary extracts on blood sugar. The posterior lobe melanophore preparation caused a similar drop in blood sugar after boiling in N/10 NaOH for 15 minutes.

Further evidence against the possibility that adrenalin secretion causes the metabolic effect was derived from study of the R.Q. Table 8 gives the R.Q. values with preparations of pars intermedia and pituitary colloid. Figure 2 clearly shows the relationship between the oxygen consumption and R.Q. at the sixth to eighth hour after injection of *A.I.F.* after varying periods of fast-

TABLE 11. EFFECT OF TRYPTIC DIGESTION AT PH 8.5 ON EXTRACT *A.I.F.*[1]

	Sex	Dose	Hours after Injection	% Change B.M.R.	R.Q. Before	R.Q. After
R51	F	1 cc.	2–4	+36	.87	.84
R51	F	1 cc.+Trypsin	2–4	+ 1	.95	1.02
			6–8	+ 5		.91
R54	F	1 cc.	2–4	+37	.72?	.78
			6–8	+ 4		.76
R54	F	1 cc.+Trypsin	2–4	+ 1	.85	.88
			6–8	+ 1		.83
R54	F	4 cc.+Trypsin	2–4	− 3	.93	1.01

[1] Frog unit less than 0.5 cc. of 1/100 dilution.

TABLE 12. EFFECT OF PITUITARY EXTRACTS ON BLOOD SUGAR[1]

Before Injection	First hour	Second hour	Third hour	Fourth hour	Fifth hour
Av. 129 (Max. 143, Min. 117)	Av. 116 (Max. 127, Min. 101)	Av. 111 (Max. 120, Min. 84)	Av. 107 (Max. 120, Min. 98)	Av. 115 (Max. 122, Min. 105)	Av. 122 (Max. 131, Min. 113)

[1] Average of 8 experiments on 8 rabbits.
Extracts used: 4 cc. anterior pituitary *A* (2); boiled at pH 10 for 15 min.
4 cc. anterior pituitary *M* (4); boiled at pH 10 for 15 min.
3 mg. Stehle's posterior pituitary melanophore (2); boiled in N/10 NaOH for 15 min.

ing (2 to 48 hours). The data obtained with this extract at the second to the fourth hour after injection show a less evident correlation due to blowing-off of CO_2. This effect may be due to the inorganic constituents, since the tryptic digest and filtrate from charcoal produced a rise in R.Q. without a significant change in oxygen consumption (table 10, 11). This extract lowered the CO_2 combining power of plasma by 11 cc. per cent (average of 8 determinations). The immediate effect on the R.Q. is more clearly demonstrated by the data obtained with *Poly-B* (an aqueous extract of acetone-treated pig pituitary at pH 5) after intravenous injection (table 13). This extract contains no ammonium sulphate and was prepared from dissected anterior lobes (1 cc. = 0.41 gm.), and may be considered relatively free of posterior lobe hormones. It lowered the CO_2 combining power of plasma by 3.5 cc. per cent (average of 5). The results show the instantaneous drop in R.Q. which is especially evident in well-fed animals. The oxygen consumption increased in both the fed and fasted animals. Adrenalin controls included in table 14 gave a very different response, as one would expect from the work of previous authors. This study was undertaken at different fasting levels to stand comparison with the results obtained with extract *Poly-B*.

Owing to the difficulty of obtaining accurate urine samples from rabbits, the effect of extract *A.I.F.* on nitrogen metabolism was studied on other species. Two groups of rats, 6 in each, were fasted 4 days and during the 4th day one group received 4 injections of 0.5 cc. of *A.I.F.*, whilst the control group received 0.5 cc. of 0.5 per cent ammonium sulphate. The results are summarized in table 15. There is no evidence of increased protein metabolism. As an index of gluconeogenesis from protein, the blood urea concentrations of rabbits and dogs were studied (tables 16, 17). There was a definite decrease in both cases, whereas 2 rabbits which were resistant to metabolic stimulation did not show a corresponding fall in blood urea.

In view of the increase in oxygen consumption, it was of interest to study the changes in rectal temperature. Studies on 5 rabbits are given in table 18. The variable degree of hyperthermia is of slow onset and outrules the possibility that a sudden change in the heat-regulating mechanism may be the cause of increased metabolism. Table 19 contains a group of data obtained with extracts which for various reasons had a negligible content of melanophore expanding substance. In no case was there a significant rise in oxygen consump-

tion. In one case a very potent preparation of prolactin produced a dramatic decrease in oxygen consumption.

DISCUSSION

The separate identity of the thermostable metabolic and thyrotropic hormones has already been discussed (2). The points of distinction are: the effect of heat, the anatomical distribution in the pituitary, the development of resistance to the thyrotropic effect without a corresponding resistance to the immediate metabolic effect, the longer latent period of the thyrotropic hormone, and, finally, lack of parallelism in the two activities in various extracts. The most potent metabolic preparations are low in growth-promoting, adrenotropic and prolactin activity. Highly purified prolactin and growth preparations are similarly almost devoid of metabolic activity under the conditions of our experiments. The growth hormone and to some extent prolactin and adrenotropic hormones are also differentiated by their susceptibility to alkaline treatment (19).

Oxytocin and vasopressin are definitely excluded by the use of purified preparations of each, by their known susceptibility to alkali at high tempera-

TABLE 13. EFFECT OF INTRAVENOUS INJECTION OF EXTRACT *Poly-B* (PIG)[1]

Serial no. and sex	Before Injection	Time in hours after injection				
		¼–2	2–4	4–6	6–8	22–24
51F	O_2 109	137	144	138	128	—
	R.Q. .89	.75	.74	.77	.76	—
51F	O_2 120	130	139	127	—	119
	R.Q. .96	.83	.80	.85	—	.81
56F	O_2 133	154	167	—	—	—
	R.Q. .92	.88	.78	—	—	—
56F	O_2 127	152	—	153	121	119
	R.Q. .98	.82	—	.83	.82	.82
57F	O_2 103	136	137	131	115	109
	R.Q. .94	.78	.77	.81	.84	.85
57F	O_2 134	—	187	171	—	105
	R.Q. 1.04	—	.81	.87	—	.86
Average of fed group	O_2 121	142	155	144	121	113
	R.Q. .955	.812	.780	.826	.807	.835
51F	O_2 110	138	136	132	127	
	R.Q. .78	.75	.75	.75	.75	
51F	O_2 93	118	121	121	121	
	R.Q. .81	.75	.77	.77	.77	
57F	O_2 102	—	144	—	135	
	R.Q. .78	—	.73	—	.72	
56F	O_2 120	127	129	131	124	
	R.Q. .79	.75	.77	.77	.76	
Average of fasted group	O_2 106	128	135	128	127	
	R.Q. .790	.750	.755	.763	.750	
58F Hypophysectomized	O_2 118	120	116	116	111	
	R.Q. .85	.77	.76	.75	.74	
58F Hypophysectomized	O_2 108	112	111	117	—	
	R.Q. 1.00	.80	.83	.79	—	

[1] Boiled at pH 7 for 1 hr. and at pH 11 for 10 min. Dose = 1 cc.

TABLE 14. INFLUENCE OF ADRENALIN ON B.M.R. AND R.Q. AT VARYING INITIAL LEVELS OF R.Q.

Serial no. and sex	Dose mg.	Time after injection, hr.	% Change B.M.R.	R.Q. Before	R.Q. After
51F	0.5	2–4	+ 2	.99	.98
51F	0.5	2–4	+16	.90	.89
		6–8	+ 5		.84
51F	0.5	2–4	+33	.84	.91
51F	1.0	2–4	+24	.73	.78
52F	0.5	2–4	+32	1.04	.96
		6–8	+14		1.01
52F	0.5	2–4	+20	.78	.89
53F	0.5	2–4	+31	.93	.91
56F	1.0	2–4	+21	.81	.81
57F	0.5	2–4	+23	.86	.86
	Average of 2–4 hr. data		+22.4	.876	.888

ture (24), and also by the extremely high concentration of the metabolic stimulant in the pars intermedia and pituitary colloid.

The close similarity of the metabolic stimulant to the melanophore-dilating hormone is striking. They have a similar anatomical distribution in the various parts of the pituitary. Both withstand the action of alkali at the temperature of the boiling-water bath. They are each adsorbed by charcoal and digested slowly by trypsin but not by pepsin. We have so far obtained no convincing evidence that the metabolic effect is potentiated by alkali. Some extracts, particularly *A.I.F.*, appear to have a more prolonged metabolic activity than their melanophore content would suggest. This may possibly be due to the associated protein causing slower absorption and utilization.

In view of the different effect of adrenalin and the pituitary metabolic principle on R.Q. and blood sugar, it is suggestive that adrenalin may neutralize the action of the melanophore hormone on melanophores and the retinal pigment of frogs (34).

The subject is further complicated by the possibility of two chromatophore hormones in the pituitary (35) acting respectively on melanophores of frogs and erythrophores of certain fish. The latter substance has been named 'intermedin' by Zondek and Krohn (27) who at the time were under the impression that two reactions were mediated by a single hormone. It is relatively susceptible to alkali and Stehle's preparation of melanophore powder is known to lose nine-tenths of its erythrophore activity after boiling in N/10 alkali for

TABLE 15. 24-HOUR URINARY NITROGEN EXCRETION OF RATS WITH 4 INJECTIONS OF 0.5 CC *A.I.F.*[1]

Second day	Injected during third day	Third day	% Change
Average of 5=145.2	2 cc. *A.I.F.*	141.2	−2.6
Average of 6=111.7	2 cc. 0.5% $(NH_4)_2SO_4$	115.2	+2.7

[1] Boiled at pH 10 for 10 minutes and pH 7 for 1 hour. Animals fasted 48 hours before injection. Nitrogen expressed in grams.

TABLE 16. CHANGES IN BLOOD UREA CONCENTRATION AFTER INTRAVENOUS INJECTION OF 1 CC. *Poly-B* EXTRACT (PIG)[1]

Serial No.	Sex	Blood urea conc. before injection mg./100 cc.	Blood urea conc. at 5th hour mg./100 cc.
51	F	41.94	37.45
56	F	42.54	35.95
57	F	39.55	35.95
52[2]	F	41.95	41.20
52*A*[2]	F	40.85	40.35

[1] Boiled at pH 7 for 1 hr. and pH 10 for 15 min. Rabbits fasted 12 hr.
[2] Two rabbits whose oxygen consumptions were not significantly increased after injection. One had middle-ear disease (52A).

15 minutes (36). This treatment caused no diminution of its metabolic activity.

A study of changes in carbohydrate metabolism with such pituitary extracts will be included in another communication from this department. For the purpose of the present discussion, it may be assumed that there is no increase in carbohydrate oxidation but that, on the contrary, it may be diminished. From the data included here, we have some evidence to suggest a diminution of protein metabolism.

The question arises whether the fall in R.Q. with the increased oxygen consumption can be interpreted as due to an increased oxidation of fat. Another theoretical possibility which must be considered is gluconeogenesis from fat (37). Soskin, et al. (38) have postulated a deficiency of this mechanism in hypophysectomized dogs to explain various experimental findings. It is unlikely that this would explain all our results for the following reasons: the R.Q. tends to fall to 0.71 but never below it; the fall in R.Q. is most marked in the well-fed animals and relatively slight in fasted animals; in one hypophysectomized rabbit the R.Q. fell without any increase in oxygen consumption. A consideration of the CO_2 excretion during the increased oxygen consumption is relevant to this discussion. The following is a summary of the increased CO_2 excretion of 4 rabbits after their first injection of pituitary extract *A.I.F.* The period of fasting varied from 2 to 56 hours.

Serial No.	Sex	Hours fasted	Hours after injection	M.R. % change	Increase CO_2 excretion in gm.
R51	F	2	2–4	+20	0.238
			6–8	+36	0.162
R53	F	14	2–4	+ 8	0.331
			6–8	+24	0.366
R52	M	24	2–4	+24	0.673
			6–8	+22	0.475
R54	M	56	2–4	+27	0.864
			6–8	+33	0.823

TABLE 17. CHANGES IN BLOOD UREA OF 2 DOGS FASTED 48 HOURS BEFORE INJECTION

Serial No.	Weight kg.	Extract	Dose cc. subcut.	Blood urea conc. before injection mg/100 cc.	Blood urea conc. at 7th hr. mg./100 cc.
8	24.2	*Poly-B* (ox) boiled at pH 7 and 11	25	18.5	13.2
7	22.0	*No. I D*	22	31.76	23.67
7	22.0	*Poly-B* (pig) boiled at pH 11 for 15 min.	24	21.00	16.00

The large increase in CO_2 excretion, particularly in the fasted animals, must be accounted for by increased oxidation. This is confirmed by the hyperthermic response with such pituitary extracts. Similarly the simple assumption of increased fat oxidation cannot completely explain the results. Calculations of R.Q. based on this assumption, and neglecting the changes in nitrogen metabolism, give (particularly in the fed group) a smaller decrease in R.Q. than that actually observed: *e.g.*, in six experiments on fasted animals, the R.Q. fell from .768 to .739 and the oxygen consumption increased 32 per cent. Such an increase in oxygen consumption due to fat oxidation should depress the R.Q. to .753. The residual fall in R.Q. must be accounted for by some other means. One explanation of this discrepancy is that during the increased oxygen consumption there is a corresponding depression of carbohydrate metabolism and possibly also of protein deamination (4). This assumption fits in with the greater fall in R.Q. of the fed, in comparison with the fasted rabbit. But the discrepancy might possibly also be explained by gluconeogenesis from fat, with a concurrent inhibition of carbohydrate transformation to fat where the R.Q. is initially high. Depression of carbohydrate utilization by thermolabile pituitary extracts has been shown by Fisher, Russell and Cori (7), Meyer, Wade and Cori (9), and Russell (10).

Hence it appears that more than one factor is operating in the altered gaseous metabolism. First an increased oxidation, presumably from fat, and secondly either a depression of carbohydrate metabolism, or increased gluconeogenesis.

TABLE 18. RECTAL TEMPERATURE (F.) AFTER INTRAVENOUS INJECTION OF 1 CC. *Poly-B* EXTRACT (PIG)[1]

Serial no. and sex	Before Inj.	Hours after Injection								
		1	2	3	4	5	6	7	8	9
51F	102.6	101.8	—	103.6	105.0	106.1	105.7	—	—	103.0
56F	102.1	102.5	—	104.1	104.3	104.6	104.2	—	—	103.1
60F	102.3	102.4	104.5	103.9	102.9	—	—	—	—	—
57F	101.5	101.0	102.1	103.0	103.3	—	104.0	—	102.8	—
61F	101.7	102.0	103.1	101.9	101.9	—	—	—	—	—
Average of 5 controls	102.6	102.7	102.7	102.7	—	102.6	102.6	—	—	—

[1] Rabbits fasted 12 to 24 hours.

The rôle of the pituitary gland in fat metabolism has been stressed by previous authors. Anselmino and Hoffmann (39), from a careful study of ketonemia after injection of anterior lobe extracts, described a fat-metabolism hormone. It had no effect on R.Q., depressed the B.M.R. (40) and was easily destroyed by heat. Raab (41) studied changes in blood fat following injection of a preparation termed 'lipoitrin', obtained from both anterior and posterior lobes. He claimed that it was a separate hormone which influenced fat absorption from the blood to the liver. Gaebler (5) suggested an increased fat oxidation from studies on gaseous metabolism and nitrogen excretion following a single large injection of an impure growth preparation made from anterior lobes.

TABLE 19. EFFECT OF VARIOUS PITUITARY EXTRACTS, LOW IN MELANOPHORE ACTIVITY, ON GASEOUS METABOLISM

Serial No.	Extract	Dose cc.	Time after injection hrs.	% Change in O_2 consumption	R.Q. Before	R.Q. After
51	Growth ♀ 10	10	2–4	+ 1	.80	.81
55	1.5 N dry ammoniacal alcohol	2	2–4	− 5	.78	.76
51	Extract 10	4	2–4	0	.75	.79
51	1.5 N dry ammoniacal alcohol	5	2–4	− 3	.84	.83
42	Prolactin (of March 5)	4	2–4	−29	—	—
21	Iso-electric precipitate =*G.A.P.* preparation	4	2–4	− 9	—	—
21	Iso-electric filtrate (*IA*) =*G.A.P.* preparation	4	2–4	+10	—	—
51	*Poly-B* pig (May, 1937)	4	2–4	+ 4	.86	.84

SUMMARY

A specific metabolic stimulant present in pituitary extracts is described; it increases the oxygen consumption of rabbits, and depresses the respiratory quotient; CO_2 production is increased, especially in fasted animals and body temperature rises; there is evidence of decreased nitrogen metabolism.

The hypothesis that fat metabolism is stimulated is advanced; but the depression of R.Q. being large relatively to the increase in oxygen consumption, it appears that there must be in addition some suppression of carbohydrate oxidation, or some increase of gluconeogenesis.

The active principle is thermostable, resistant to alkali and to pepsin, destroyed by trypsin, and adsorbed upon charcoal. In these respects, and in its distribution in different parts of the pituitary, and in its occurrence in extracts of various types, it exactly resembles the melanophore-expanding hormone. Reasons are given for distinguishing the active principle from the thyrotropic, adrenotropic and growth hormones of the anterior lobe, and the pressor and oxytocic substances of the posterior lobe. Active extracts have been prepared from ox, sheep and pig pituitaries; the active principle is present in both anterior and posterior lobes but has been found in highest concentration in pituitary colloid and in extracts of the pars intermedia.

The authors wish to express their gratitude to Dr. R. L. Stehle of the Department of Pharmacology, McGill University, for his generosity in supplying us with samples of melanophore preparation; also to Dr. E. B. Astwood of the Johns Hopkins Hospital for extracts of pars intermedia and pituitary colloid; and to Dr. E. M. K. Geiling of the Department of Pharmacology, University of Chicago, for a specimen of anterior lobe of whale pituitary. We also wish to acknowledge the helpful advice and criticism of Dr. D. L. Thomson during the course of this work.

REFERENCES

1. O'Donovan, D. K.: Am. J. Physiol. **119:**381. 1937.
2. O'Donovan, D. K., and J. B. Collip: Tr. Am. A. Study Goiter, 1937.
3. Houssay, B. A., and A. Artundo: Compt. rend. Soc. de biol. **114:**391. 1933.
4. Gaebler, O. H.: Am. J. Physiol. **110:**584. 1935.
5. Gaebler, O. H.: J. Exper. Med. **57:**349. 1933.
6. Riddle, O., G. C. Smith, R. W. Bates, C. S. Moran and E. L. Lahr: Endocrinology **20:**1. 1936.
7. Fisher, R. E., J. A. Russell and C. F. Cori: J. Biol. Chem. **115:**627. 1936.
8. Fisher, R. E., and R. I. Pencharz: Proc. Soc. Exper. Biol. & Med. **34:**106. 1936.
9. Meyer, H. S., L. J. Wade and C. F. Cori: Proc. Soc. Exper. Biol. & Med. **36:**346. 1937.
10. Russell, J. A.: Am. J. Physiol. **121:**756. 1938.
11. Krogh, M., and H. Okkels: Compt. rend. Soc. de biol. **112:**1694. 1933.
12. Diefenbach, O. L.: Endokrinologie **12:**250. 1933.
13. Zunz, E., and J. LaBarre: Compt. rend. Soc. de biol. **118:**1622. 1935.
14. Burn, J. H., and H. W. Ling: J. Physiol. **65:**191. 1928.
15. Hagedorn, H. C., and B. N. Jensen: Biochem. Ztschr. **135:**46. 1923.
16. Van Slyke, D. D., and G. E. Cullen: J. Biol. Chem. **30:**289. 1917.
17. Van Slyke, D. D., and G. E. Cullen: J. Biol. Chem. **24:**117. 1916.
18. Anderson, E. M., and J. B. Collip: J. Physiol. **82:**11. 1934.
19. Collip, J. B.: Cold Spring Harbor Symposia on Quantitative Biology, Vol. 5, p. 210, 1937.
20. Stehle, R. L.: J. Pharmacol. & Exper. Therap. **57:**1. 1936.
21. Stehle, R. L.: J. Biol. Chem. **102:**573. 1933.
22. Geiling, E. M. K., and A. DeLawder: Johns Hopkins Hosp. Bull. **51:**335. 1932.
23. Grollman, A., and E. M. K. Geiling: J. Pharmacol. & Exper. Therap. **46:**447. 1932.
24. Hogben, L. T., and C. Gordon: Brit. J. Exper. Biol. **7:**286. 1930.
25. Jores, A.: Ztschr. f. exper. Med. **87:**266. 1933.
26. Lewis, D., F. C. Lee and E. B. Astwood: Johns Hopkins Hosp. Bull. **61:**198. 1937.
27. Zondek, B., and H. Krohn: Klin. Wchnschr. **11:**405, 849, 1293. 1932.
28. Noether, P.: Klin. Wchnschr. **11:**865. 1932.
29. Valsö, J.: Klin. Wchnschr. **13:**1819. 1934.
30. Geiling, E. M. K.: Johns Hopkins Hosp. Bull. **57:**123. 1935.
31. Jores, A.: Arch. f. exper. Path. u. Pharmakol. **173:**31. 1933.
32. Dietel, F. G.: Klin. Wchnschr. **12:**1683. 1933.
33. Hogben, L. T., and F. R. Winton: Proc. Roy. Soc., Lond. B, **93:**318. 1922.
34. Jores, A., and K. G. Caesar: Arch. f. d. ges. Physiol. **235:**724. 1935.
35. Jores, A., and G. Will: Ztschr. f. d. ges. exper. Med. **94:**389. 1934.
36. Stehle, R. L.: Ergebn. d. Vitamin- u. Hormonforsch. **1:**318. 1938.
37. Cathcart, E. P., and J. Markowitz: J. Physiol. **63:**309. 1927.
38. Soskin, S., I. A. Mirsky, L. M. Zimmerman and N. Crohn: Am. J. Physiol. **114:**110. 1935.
39. Anselmino, K. J., and F. Hoffmann: Klin. Wchnschr. **10:**2380. 1931.
40. Hoffmann, F., and K. J. Anselmino: Klin. Wchnschr. **10:**2383. 1931.
41. Raab, W.: Klin. Wchnschr. **13:**281. 1934.

STUDIES OF THE EFFECTS OF PITUITARY EXTRACTS ON CARBOHYDRATE AND FAT METABOLISM

A. H. NEUFELD AND J. B. COLLIP
From the Department of Biochemistry, McGill University
MONTREAL, CANADA

The effects of various pituitary extracts on carbohydrate and fat metabolism have been the subject of numerous studies since Houssay (1) demonstrated the ameliorating effect of hypophysectomy upon pancreatic diabetes. The more recent work of Long and his collaborators (2), calling attention to the importance of the adrenal cortex, served also to stimulate further investigations upon this problem.

There have been so many physiological effects described for anterior lobe principles that the question is always before us as to the number of anterior lobe principles and the functions of each. Until these various principles have been obtained in much purer form than has been possible until now, it is obviously impossible to be certain that two or more of the well known effects of anterior lobe extracts are due to a single hormone substance. The experiments which are reported in this communication represent an attempt to simplify the whole question of the multiplicity of pituitary hormones. Evidence is presented which, though incomplete, at least suggests very strongly that the ketogenic effect and the apparent antagonism to adrenalin hyperglycemia and insulin hypoglycemia as seen in rabbits are due to one principle. Moreover, there is suggestive evidence that the specific metabolic stimulant action described in detail in another communication, the chromatophore-expanding action of pituitary extracts as seen in the frog (3), and the glycostatic effect, may all be due to the same principle. This presumably multipotent substance is very resistant to exposure in aqueous solution to high temperatures over a wide pH range. It is readily dialyzable through various membranes, and it can be recovered by electrodialysis in the cathode cell (4). These various properties which have been mentioned seem to run more or less parallel with the melanophore content of extracts, and in point of origin the active substance is most likely the pars intermedia.

MATERIALS AND METHODS

The first part of this work was confined to a study of the ketogenic properties of pituitary preparations. For the study of the urinary excretion of total acetone bodies, determined by the gravimetric method of Van Slyke (5), the rats were kept in Hopkins metabolism cages; the urine was collected separately from the feces and preserved in copper sulphate solution. Male rats, weighing between 75 and 100 gm., fasted for 24 hours and kept at a temperature of 30°C. with a controlled water and salt intake, were used for the work. Blood-

acetone bodies were determined by the method of Van Slyke and Fitz (6). The following method was adopted as the standard procedure for the determination of the ketogenic response of extracts by means of the blood-acetone curve.

Sixteen male rats, weighing between 120 and 160 gm., were fasted for 24 hours. At the end of this period they were given food (powdered Purina) for 3 hours. By this means an approximately equal intake of food by the individual animal was ensured. Sixteen hours after the removal of food, 8 animals received the extract under consideration by subcutaneous injection; the other 8 were given the same amount of a control preparation. On the third, sixth, ninth and twelfth hours after injection, 2 experimental and 2 control animals were bled. The time of analysis was varied with certain extracts, depending on the type of response observed.

Alkaline extracts of beef pancreas and pineal glands, prepared according to the method of Burn and Ling (7) were used as control preparations. Both extracts were found to possess no ketogenic activity. Rabbits, guinea pigs and rats were used to determine the effect of extracts upon muscle and liver glycogen. Rabbits were used also for a study of the effects of extracts upon blood sugar. The rabbits and guinea pigs were bred and supplied by the same dealer. They were given a standard feed consisting of clover hay, rolled oats and cabbage.

Liver and muscle samples for glycogen determinations were secured under amytal anesthesia. The method outlined by Bachman and Toby (8) was used for rabbits, while the method used by Collip, Thomson and Toby (9) was used for rats and guinea pigs. Glycogen determinations were carried out by the method of Good, et al. (10); the modified Shaffer-Hartman method described by Peters and Van Slyke (11) was used to determine the sugar formed as well as the blood sugar estimations. In the tables glycogen is expressed as glucose. The liver and blood neutral fat represents the fraction soluble in both hot alcohol-ether and cold chloroform.

Two Houssay dogs, operated on several months previously, were used. Both had been on a constant food intake, consisting of 250 gm. of lean meat and 50 gm. of raw pancreas, twice daily, along with 200 cc. of milk.

The pituitary extracts used were prepared as follows.

a) Preparation *B* was an alkaline extract of fresh beef anterior pituitary lobes prepared according to the method of Burn and Ling (7).

b) Preparation *A.I.F.* This extract was the same as was used extensively by O'Donovan and Collip in metabolic studies and is described in detail in their paper (4).

c) Extract *622* was prepared by heating on a boiling-water bath, for 1 hour, the primary aqueous extract (pH 5) of dissected anterior lobes of hog pituitaries which had previously been dehydrated and defatted with acetone and extracted with alkaline alcohol (pH 9, universal indicator). A protein precipitate which formed was filtered off and the filtrate was concentrated until 1 cc. represented 2 gm. of original tissue; 25 per cent NH_4OH water was added to make 2 per cent NH_4OH in the solution. After standing for 1 hour

at room temperature, the precipitate which had formed was removed and the solution was heated for 1 hour on a boiling-water bath. The ammonia was then removed by vacuum distillation and the final extract was put into ampoules; 0.1 per cent tricresol was added as a preservative. The ampoules were heated in an Arnold sterilizer for 20 minutes before capping.

d) The dialysate of *622* was prepared by dialyzing the extract through a collodion membrane against distilled water for 36 hours with 6 changes of water. The combined dialysates were concentrated under diminished pressure to the original volume.

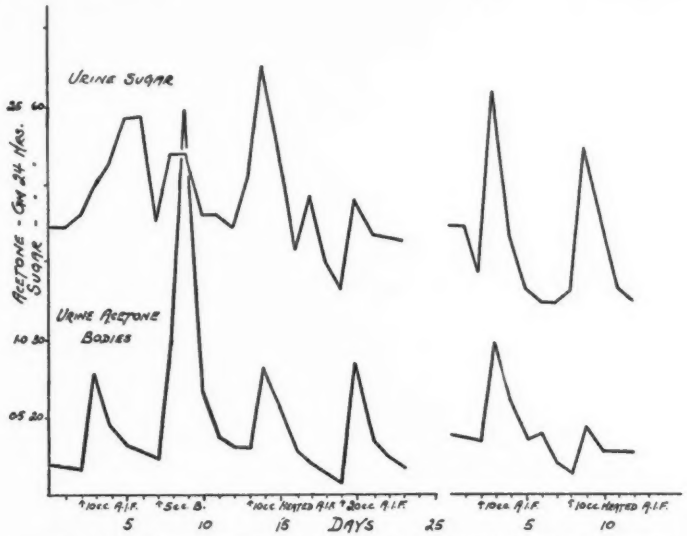


Fig. 1. The effect of pituitary preparations on glycosuria and ketonuria in a pancreatectomized-hypophysectomized dog (the heated *A.I.F.* was kept in a boiling-water bath, first at pH 7 for 1 hour, then at pH 11 for 10 minutes).

e) Extract *P.P.E.* was a posterior pituitary extract prepared according to the directions of the American Pharmacopoeia. It was heated on a boiling-water bath for 1 hour at pH 7 and finally for 10 minutes at pH 11. One cubic centimeter of the final preparation represented the equivalent of 0.5 gm. of original gland.

RESULTS

The Effect of Anterior Pituitary Extracts on Urine Acetone Bodies. The ketogenic effect of certain anterior pituitary extracts on the acetone bodies excretion in fasted rats or animals on a high fat intake has been demonstrated by a number of authors. We have had great difficulty in obtaining uniform results in this ketosis test. Even when such factors as environmental temperature, age of the animal and fluid intake were controlled, wide variations in response were noted. Thus 1 cc. of extract *B*, which we have used as a standard preparation, showed a response, in a group of 24 rats, varying from 7 to 95 mg. total acetone bodies in terms of acetone per 24 hours for 100 gm. of rat.

In the study of the ketonuria in adrenalectomized rats treated with anterior pituitary extracts, results similar to those of Long and Lukens (12), Frey (13) and MacKay and Barnes (14) were obtained. It should be noted, however, that about one of every six tested rats showed what might be considered a normal response, although no adrenal tissue was observed in these animals on post-mortem examination.

TABLE 1. THE EFFECT OF PITUITARY PREPARATIONS ON KETONURIA IN NORMAL RATS

Control	Extract *B* 1 cc. subcutaneously	*A.I.F.* 2 cc. subcutaneously	Heated *A.I.F.*[1] 2 cc. subcutaneously
	mg. acetone per 100	*gm. of rat in 24 hr.*	
0.75	23.5	2.05	1.0
0.4	25.3	1.1	1.9
0.6	46.5	4.9	3.5
0.5	12.6	1.95	2.9
0.4		4.9	1.0
0.5			

[1] In boiling-water bath, first at pH 7 for 1 hour, then at pH 11 for 10 minutes.

We have found that the totally pancreatectomized dog maintained on insulin and the pancreatectomized-hypophysectomized dog are excellent test objects for the ketogenic substance, although not as convenient as the normal rat. The effects of the various pituitary preparations on the excretion of acetone

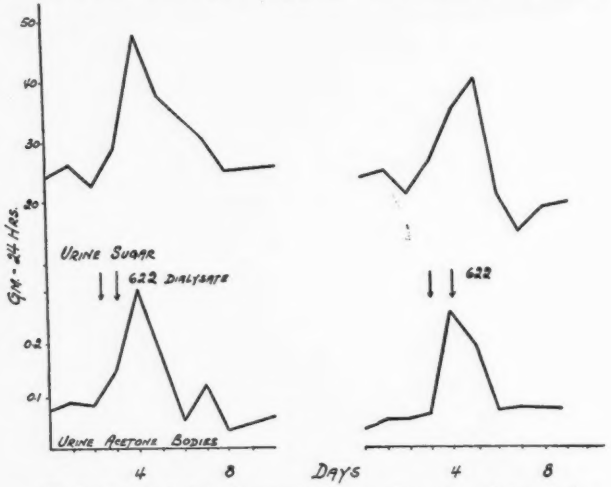


Fig. 2. The effect of pituitary preparations on glycosuria and ketonuria in a pancreatectomized-hypophysectomized dog (arrows indicate days of injections, 3 cc. 3 times daily).

bodies in Houssay dogs are shown in figures 1 and 2, and a comparison of the ketonuria produced in normal rats by extracts *B* and *A.I.F.* in table 1.

The Effect of Pituitary Extracts on Blood Acetone Bodies. We have found that the blood acetone curve is the most satisfactory method of determining the ketogenic activity of extracts. The ketonemic action of a number of different extracts is shown in table 2.

We have carried out a series of experiments to determine the ketonemic response of pituitary extracts in adrenalectomized rats. A typical result obtained with a group of rats 2 days following adrenalectomy is shown in figure 4. All adrenalectomized rats were given saline following the operation. In a

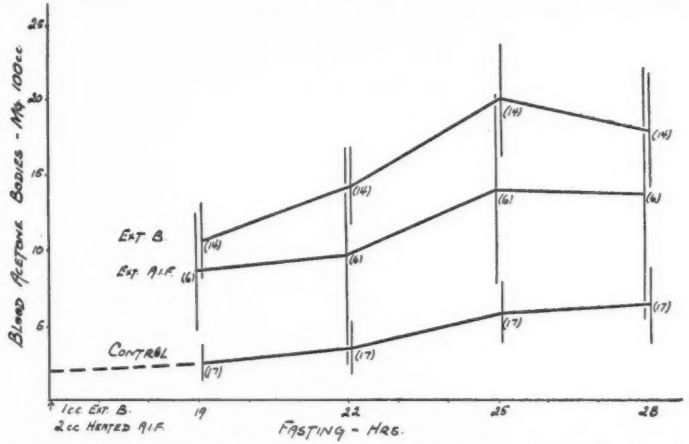


Fig. 3. The ketonemic response to pituitary preparations in normal rats (the figures in parentheses show the number of analyses, vertical lines the deviations). (Heated *A.I.F.* was kept in boiling-water bath at pH 7 for 1 hour and pH 11 for 10 minutes).

TABLE 2. THE KETONEMIC ACTION OF PITUITARY PREPARATIONS IN RATS

	Amount inj. cc.	Hours following treatment Blood acetone bodies in mg. per 100cc.			
		3	6	9	12
Control		2.5±1.3(17)[2]	3.5±1.8(21)	5.8±2.1(17)	6.4±2.5(17)
Heated *A.I.F.*[1]	2	8.6±4.0(6)	9.7±7.3(6)	14.0±6.4(6)	13.7±8.4(6)
Heated *B*[1]	1	8.6±4.0(6)	8.2±7.7(6)	14.2±6.9(6)	16.2±6.2(6)
Norite-extracted *A.I.F.*	2	3.1±2.2(3)	0.6±4.0(3)	3.7±2.2(3)	—
A.I.F. by mouth	6	1.4±1.1(3)	1.3±1.5(3)	2.4±1.8(3)	6.4±4.2(3)
B by mouth	6	1.2±1.6(3)	1.3±1.1(3)	2.7±3.7(3)	4.0±5.7(3)
622	4	5.5±4.2(3)	17.4±3.2(3)	8.2±3.2(3)	—
622 dialysate	4	5.7±5.1(3)	15.5±5.1(3)	6.4±4.2(3)	—
B in thyroidectomized	1	6.4±5.1(3)	13.3±3.5(3)	15.9±1.6(3)	20.6±3.1(3)
Adrenalin	0.5 mg./kg.	10.7±1.0(2)	5.6±1.6(2)	3.3±1.6(2)	5.8±0.7(2)
2–4 dinitrophenol	30 mg./kg.	8.4±1.8(2)	10.6±1.8(2)	10.1±1.4(2)	14.6±2.4(2)

[1] In boiling-water bath, first at pH 7 for 1 hour, then at pH 11 for 10 minutes.
[2] The figures in parentheses indicate the number of animals used.

second experiment, 8 days following adrenalectomy, similar results to those shown in figure 4 were obtained.

The fasting ketonemia of adrenalectomized rats is much higher than that of normals. After a longer fast the ketonemia produced by pituitary extracts and also the fasting ketonemia are reduced considerably. Thus in an experiment following a 36-hour fast, at which stage the animals were in very poor condition, only an insignificant ketonemic response was observed.

The Antagonistic Action of Pituitary Extracts on Insulin Hypoglycemia and Adrenalin Hyperglycemia. The effect of posterior pituitary extracts in preventing hypoglycemia following insulin injections has been demonstrated by Burn (15) and others. Stenström (16) and Burn (15) have shown that posterior pituitary extracts will also antagonize the hyperglycemic action of adrenalin. On the other hand, various authors (17, 18, 19, 20) have described an anti-insulin action of anterior pituitary preparations. The question naturally arises whether there is an essential difference between the anti-insulin effect of anterior and posterior lobe extracts.

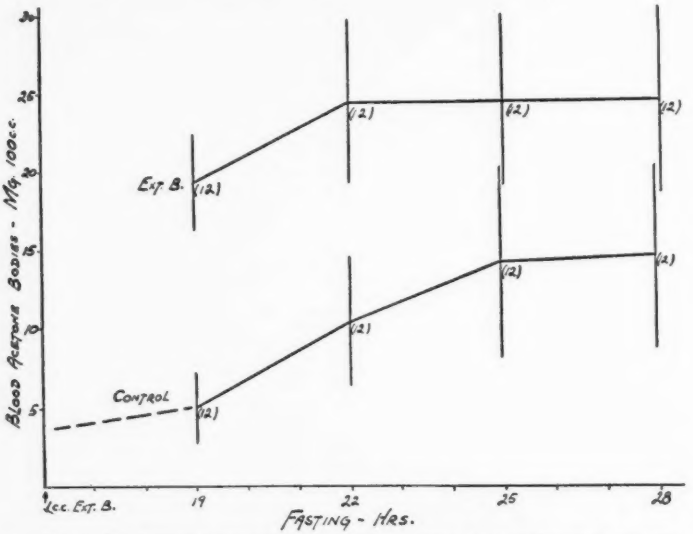


Fig. 4. The ketonemic response to pituitary preparations in adrenalectomized rats (the figures in parentheses show the number of analyses, vertical lines the deviations).

Tables 3 and 4 show the effects of extract *622* and its dialysate and extract *P.P.E.* in preventing insulin hypoglycemia and adrenalin hyperglycemia. Apparently the factors producing these effects are either closely related, or identical. The results also show that a more pronounced antagonism to insulin hypoglycemia and adrenalin hyperglycemia resulted after prolonged pretreatment; the adrenalin antagonism appeared to be more defined than that to insulin.

The Effect of Pituitary Extracts on Blood and Urine Sugar. Both hypoglycemia and hyperglycemia have been observed following treatment with certain anterior lobe preparations. Anselmino and associates (21) described a pancreatropic hormone which, in addition to producing changes in islet tissue, caused a lowering of blood sugar. Houssay and his coworkers (1) clearly demonstrated the existence of a blood-sugar-raising principle in anterior lobe tissue.

We have observed a variable response in normal rabbits to pituitary preparation, as is shown in table 5. Of the 17 animals tested, 6 showed a hyperglycemic response, while the remaining 11 showed no effect.

TABLE 3. THE ANTAGONISTIC ACTION OF PITUITARY EXTRACTS ON INSULIN HYPOGLYCEMIA IN RABBITS (FASTED 24 HOURS)

Rabbit	Body Wt. kg.		Insulin Dosage u	Control Blood Sugar	Blood sugar following insulin (hr.)				
					1	2	3	4	6
					mg. per 100 cc.				
1	3.05	Control, insulin only	6	111	39	39	convulsions		
		622 dialysate, *a*)	6	100	92	83	79	92	119
2	2.75	Control, insulin only	6	103	62	50	71	83	107
		622 dialysate, *a*)	6	87	96	96	87	107	100
3	3.63	Control, insulin only	6	92	50	33	39	54	87
		622, *b*)	6	97	97	73	77	72	64
4	4.08	Control, insulin only	6	87	67	54	50	44	83
		622, *b*)	6	89	106	102	106	89	85
5	1.60	Control, insulin only	6	100	58	33	44	50	79
		622, *c*)	6	95	95	82	82	82	87
6	3.00	Control, insulin only	6	103	58	33	39	62	75
		622, *c*)	6	97	102	85	81	85	93
	3.20	*622*, dialysate, *g*)	6	111	54	44	50	54	83
7	2.10	Control, insulin only	6	115	39	27	convulsions		
		622, *d*)	6	96	58	62	62	58	50
8	2.08	Control, insulin only	6	107	33	27	convulsions		
		P.P.E., *d*)	6	100	71	62	62	58	54
9	2.20	Control, insulin only	6	92	33	33	convulsions		
		622, *e*)	6	103	67	54	44	50	62
		622, dialysate, *g*)	6	103	67	62	54	50	75
10	1.65	Control, insulin only	6	103	33	27	convulsions		
		622, *e*)	6	100	62	50	44	50	58
18	2.30	Control, insulin only	9	103	33	33	convulsions		
		622, *f*)	9	92	67	50	27	convulsions	
19	2.13	Control, insulin only	9	103	36	33	convulsions		
		622, *f*)	9	92	92	71	33	33	33
20	2.98	Control, insulin only	9	96	42	33	convulsions		
		622, *f*)	9	103	79	62	33	33	33

a). 4 cc. 1, 1 and 2 cc. subcutaneously, 16, 10 and 1 hr. respectively before insulin.
b). 3 cc. 1 and 2 cc. subcutaneously, 12 and 1 hr. respectively before insulin.
c). 3 cc. 1, 1 and 1 cc. subcutaneously, 16, 3 and 1 hr. respectively before insulin.
d). 2 cc. 1 and 1 cc. subcutaneously, 2 and 1 hr. respectively before insulin.
e). 1.5 cc. 1 and 0.5 cc. intravenously, 10 and 1 hr. respectively before insulin
f). 5 cc. 2 and 3 cc. subcutaneously, 12 and 1 hr. respectively before insulin.
g). 9 cc. 3, 3 and 3 cc. subcutaneously, 15, 2 and 1 hr. respectively before insulin.

The administration of the heat-stable and dialyzable factor to the pancreatectomized-hypophysectomized dog, kept on a carefully controlled food intake, produced an increased glycosuria and ketonuria, as shown in figures 1 and 2.

The Effect of Pituitary Extracts on Liver and Muscle Glycogen. Russell and Bennett (22) have described a glycostatic factor in pituitary preparations that will maintain muscle glycogen of hypophysectomized rats at normal or supra-normal levels during fasting. More recently, Young (23) has described a glycotropic action of anterior pituitary extracts which, in fasted rabbits, pro-

TABLE 4. THE ANTAGONISTIC ACTION OF PITUITARY EXTRACTS ON ADRENALIN HYPERGLYCEMIA IN RABBITS

Rabbit	Body Wt. kg.		Adrenalin dosage mg./kg.	Control Blood Sugar	Blood sugar following adrenalin (hr.)				
					1	2	3	4	6
		Fasted 24 hr.			*mg. per 100 cc.*				
7	2.10	Control, adrenalin only	0.05	103	157	174	96	83	87
		622, dialysate, *a*)	0.05	111	124	119	96	87	87
8	2.08	Control, adrenalin only	0.05	103	183	128	103	79	79
		622, *a*)	0.05	107	115	107	75	87	92
14	2.40	Control, adrenalin only	0.1	111	239	346	256	174	132
		622 dialysate, *f*)	0.1	103	136	136	141	153	124
		P.P.E., *b*)	0.1	100	107	128	136	119	96
15	2.30	Control, adrenalin only	0.1	107	157	190	161	153	132
		622, *b*)	0.1	107	92	87	92	96	92
	2.40	*622*, *f*)	0.1	103	103	111	111	107	107
1	3.05	Control, adrenalin only	0.2	109	330	350	317	211	92
		622, *c*)	0.2	103	124	136	128	119	96
		622 dialysate, *f*)	0.2	107	119	157	166	170	141
		Not Fasted							
3	3.68	Control, adrenalin only	0.25	103	272	321	305	166	87
		622, *d*)	0.25	111	153	248	215	182	190
4	3.93	Control, adrenalin only	0.25	107	301	432	420	325	186
		622, *e*)	0.25	107	305	350	412	383	301

a). 1.5 cc., 0.5 and 1.0 cc. subcutaneously, 10 and 1 hr. respectively before adrenalin.
b). 3.5 cc., 1.5, 1 and 1 cc. subcutaneously, 2 and 1 hr. before and 1 hr. after adrenalin resp.
c). 2.0 cc., 0.5 and 1.5 cc. subcutaneously, 12 and 1 hr. respectively before adrenalin.
d). 2.0 cc., 1.5 cc. subcutaneously and 0.5 cc. intravenously, 12 and 1 hr. respectively before adrenalin.
e). 1.0 cc., 0.5 and 0.5 cc. intravenously, 1 hr. before and 1 hr. after adrenalin respectively.
f). 5.0 cc., 2, 2 and 1 cc. subcutaneously, 16 and 1 hr. before and 1 hr. after adrenalin resp.

duces an increase in liver glycogen, and at the same time abolishes the hypoglycemic action of insulin.

Preliminary experiments (table 6) suggest that the heat-stable factor in our pituitary preparations is probably identical with the glycostatic factor of Russell and Bennett and the glycotropic factor of Young. However, our results with fasted rabbits (table 7) are not as uniform as those reported by Young.

DISCUSSION

The question as to whether the ketogenic effect of these purified heat and alkali treated extracts is due to the same substance which is present in simple alkaline extracts of fresh gland is difficult to answer. It has been our experience, like that of others, that boiling inactivates simple extracts. On the other hand, the potency of simple extracts, as far as the production of ketosis is concerned, is many times greater, per unit of original tissue, in primary extracts than it is in such extracts as *622*. We cannot say as yet what the effects of prolonged treatment with large doses of *622* will be, but judging from preliminary tests, it is definitely not diabetogenic in the normal animal. It is possible that there are two factors involved in ketosis, and that one is thermolabile (2). The protein content of extracts is a most important factor as re-

gards the degree of apparent destruction which occurs on boiling an extract as well as the degree of activity which is manifested after subcutaneous injection. The ketogenic effect of an extract rich in protein is always much more prolonged than is that obtained with *622*. High protein content in general prolongs the effect after injection and slows down or stops dialysis through membranes, probably by actual closure of the pores. It is of interest to note here that in our experiments on the Houssay dog, in which a comparison was made between the increased output of acetone bodies and of sugar induced by different extracts, the crude alkaline extract had a relatively greater effect upon the former than upon the latter (fig. 1).

The only data available in the literature on the production of ketonemia with pituitary extracts in fasted adrenalectomized rats are those of MacKay and Barnes (14) on 4 animals. Variations in the conditions of the test make a direct comparison with our own results difficult. Our results indicate that the ketonemia of the adrenalectomized rats treated with potent ketogenic extracts is similar to that of normals, provided the animals are in good condition. The failure of adrenalectomized rats to show ketonuria, except in rare instances, suggests that the kidney threshold for acetone bodies must be raised.

The fact that an extract of pituitary tissue which has been subjected to such rigorous treatment, as for example preparation *622,* is still capable of producing several definite physiological effects suggests that a single substance is responsible for all of these. Our colleague, Dr. O. F. Denstedt, has shown (3) that further purification by electrodialysis can be obtained of the fraction causing ketonemia in fasted rats, and having an antagonistic action on the hyperglycemic effect of adrenalin and the hypoglycemic action of insulin.

Since the growth, thyrotropic, gonadotropic and adrenotropic effects of anterior lobe extracts can be differentiated from this fraction, and since the

TABLE 5. THE EFFECT OF EXTRACT *622* ON THE BLOOD SUGAR OF NORMAL RABBITS

Rabbit	Length of fast hr.	Amount injected cc.	Control Blood Sugar	Blood sugar following injection (in hr.)						
				1	2	3	4	6	8	24
				mg. per 100 cc.						
1	16	3 subcutaneously	96		96		92	92	75	124
2	16	3 subcutaneously	107		219		398	522	534	145
3	16	2 subcutaneously	96			119	204	96	83	96
4	16	2 subcutaneously	87			87	83	83	92	83
1	0	3 subcutaneously	115	132	153		132	141	149	119
2	0	3 subcutaneously	115	132	132		153	178	186	121
3	0	6 subcutaneously	115		207		149	153	136	
4	0	6 subcutaneously	128		107		107	104	104	
7	24	3 subcutaneously	104	100	96	100	100	92		
8	24	3 subcutaneously	107	100	104	104	96	92		
9	24	3 subcutaneously	104	136	136	103	92	100		
10	24	3 subcutaneously	104	100	96	96	92	92		
11	24	1 subcutaneously	100	87	83	83	83	87		
12	24	1 intravenously	96	96	103	87	92	92		
18	24	1 intravenously	96	100	83	87	79	83		
19	24	1 intravensouly	103	111	96	92	79	92		
20	24	1 intravenously	107	111	100	87	83	87		

melanophore-expanding property of the tissue of the pars intermedia seems to parallel the degree of potency of extracts as regards a number of miscellaneous physiological properties, such as those described in this paper as well as the specific metabolic effect demonstrated by O'Donovan and Collip (4), it may be concluded that the substance or substances responsible for these effects have their origin in the pars intermedia.

Because of the relatively great stability of the active principle causing the ketonemic response, it was thought that it might possibly be effective when administered orally. The results of the oral test were completely negative (table 2).

Thyroidectomized rats respond in a normal manner to the *622* preparation (24).

Muscle glycogen of normal rats and guinea pigs, either fasted or fed and treated with the heat-stable factor, showed in the majority of cases a tendency to increase. In hypophysectomized rats, following an 8-hour fast, such preparations maintained the muscle glycogen at normal levels. The liver glycogen values in such animals remained low, although definitely higher than in untreated controls. Attempts to produce abnormally high glycogen levels in fed hypophysectomized rats failed.

Rabbits treated with the thermostable factor showed in the majority of cases one of two types of response. Some animals showed supra-normal levels of liver and muscle glycogens with a normal or sub-normal liver fat, but with no apparent change in the blood fat and ketone bodies. Others, however, showed varying degrees of fatty infiltration of the liver, frequently resulting in an increased lipoid content.

The relatively high glycogen values observed in some of the treated animals suggest that new sugar had been formed. Gluconeogenesis from protein, however, is improbable since it has previously been shown (4) that the blood

TABLE 6. CARBOHYDRATE LEVELS IN RATS AND GUINEA PIGS TREATED WITH ANTERIOR PITUITARY EXTRACTS

		No. of Animals	Liver Glycogen mg. %		Muscle Glycogen mg. %	
			Aver.	Range	Aver.	Range
I	Guinea pigs, fasted 24 hr.					
	1. Untreated controls	4	875±130	770–1050	522± 44	478– 574
	2. *622*, 4 cc. in 24 hr.	7	1620±362	970–2810	664± 94	520– 766
II	Guinea pigs, fasted 48 hr.					
	1. Untreated controls	3	143± 75	90– 230	580± 90	490– 670
	2. *622*, 2 cc. per day, 2 days	3	560±219	380– 800	814± 41	770– 852
III	Rats, not fasted					
	1. Untreated controls	5	3342±797	2350–4150	506± 30	479– 558
	2. *622*, 2 cc. per day, 7 days	5	654±138	520– 900	1031±474	477–1721
IV	Hypophysectomized rats, fasted 8 hr.					
	1. Untreated controls	9	19± 7	12– 33	406± 93	279– 560
	2. *622* dialysate, 1 cc. per day, 4 days	9	511±509	124–1330	518± 60	438– 617

TABLE 7. THE EFFECT OF PITUITARY EXTRACTS ON 22-HOUR FASTED RABBITS

Sex and Weight kg.	Control Blood			Blood Sugar		Final Blood		Liver Neutral fat	Liver glycogen	Muscle glycogen
	Sugar mg./100 cc.	Neutral fat gm./100 cc.	Acetone bodies mg./100 cc.	Before anesthesia mg./100 cc.	After anesthesia mg./100 cc.	Neutral fat gm./100 cc.	Acetone bodies mg./100 cc.	%	%	%
Control, Amytal Anesthesia										
M 3.55								6.07	0.29	0.455
F 3.55								6.01	2.35	0.639
5 cc. 622 Dialysate Twice in 22 Hours										
M 2.28					119	0.59	3.3	3.67	13.39	0.852
M 2.95					132	0.69	3.1	4.71	13.21	1.254
M 2.35								7.62	13.05	0.826
M 2.35								6.97	9.58	0.879
F 2.35	107	0.73	2.4	119	145	1.51	8.4	11.62	9.00	1.216
F 2.55								16.02	0.25	0.601
M 1.88	103	0.67	3.9	111	174	0.71	4.0	6.00	8.32	0.468
M 3.20								6.85	2.93	0.554
6 cc. 622 Dialysate Twice in 22 Hours										
F 2.05	115	0.75	4.2	111	153	3.16	10.0	11.65	4.89	0.482
F 2.10	107	0.71	2.9	138	141	0.61	1.3	9.77	5.49	0.551
M 1.80	111			115	149	0.71	0.0	8.52	4.03	0.498
8 cc. 622 Dialysate Twice in 22 Hours										
M 4.05	128	0.93	0.4	75	100	1.52	10.6	12.64	1.52	0.575
F 4.20	115	0.71	3.8	92	124	0.90	10.4	10.68	2.58	0.602
M 2.25	103			115	132	0.80	2.4	4.74	6.42	0.508
M 2.05	111			132	153	0.78	3.3	5.16	6.37	0.531

urea in rabbits and dogs, treated with similar preparations, remained constant or decreased. This is further supported by the finding that the excessive glycosuria and ketonuria produced by the heat-stable and dialyzable factor in the Houssay dog is not associated with an increased nitrogen excretion.

In an attempt to establish the relation of the heat-stable factor to the diabetogenic substance (1, 25, 26) in pituitary preparations, we treated a 7-kg. male dog, kept on a meat diet, with the *622* preparation for 8 weeks. The weight of the animal remained unchanged and no hyperglycemia, glycosuria or ketonuria was at any time detected. At the end of this period the diet of the animal was changed to bread and milk, the injections being continued. The dog gained 2 kg. in body weight within the next 7 days. Three weeks later the animal was sacrificed for analysis following a 10-hour fast. At this time he weighed 9.1 kg. The results obtained were: liver glycogen 7.23 per cent, muscle glycogen 1.12 per cent, heart muscle glycogen 0.894 per cent and liver neutral fat 4.70 per cent. An excess of depot fat, especially in the peritoneum, was found. Histologically the pancreas appeared normal.

SUMMARY

Results reported herein indicate that there is obtainable from pituitary tissue a fraction which is thermostable within a wide range of pH. This fraction has been demonstrated to have the following physiological properties. *a)*. Production of ketonemia in both normal and adrenalectomized rats. *b)*.

Production of increased glycosuria and ketonuria in the Houssay dog. *c)*. An antagonistic action on the hypoglycemic action of insulin, and on the hyperglycemic action of adrenalin. *d)*. A glycostatic action which has been demonstrated in rats, guinea pigs and rabbits.

REFERENCES

1. Houssay, B. A.: Dunham Lectures, New England J. Med., p. 214. 1936.
2. Long, C. N. H.: Harvey Lectures, p. 194. 1936-37.
3. Denstedt, O. F., and J. B. Collip: Canad. M. A. J. **39:**84. 1938.
4. O'Donovan, D. K., and J. B. Collip: Endocrinology **23:**718. 1938.
5. Van Slyke, D. D.: J. Biol. Chem. **32:**455. 1917.
6. Van Slyke, D. D., and R. Fitz: J. Biol. Chem. **39:**23. 1919.
7. Burn, J. H., and H. W. Ling: J. Physiol. **65:**191. 1928.
8. Bachman, C., and G. Toby: J. Physiol. **87:**1. 1936.
9. Collip, J. B., D. L. Thomson and G. Toby: J. Physiol. **88:**191. 1936.
10. Good, C. A., H. Kramer and M. Somogyi: J. Biol. Chem. **100:**485. 1933.
11. Peters, J. P., and D. D. Van Slyke: Quantitative Clinical Chemistry, Baltimore. 1932.
12. Long, C. N. H., and F. D. W. Lukens: Proc. Soc. Exper. Biol. & Med. **32:**392. 1934.
13. Frey, E. J.: Am. J. Physiol. **116:**55. 1936.
14. Mackay, E. M., and R. H. Barnes: Am. J. Physiol. **118:**184, 525. 1937.
15. Burn, J. H.: J. Physiol. **57:**318. 1923.
16. Stenström, T.: Biochem. Ztschr. **63:**472. 1913.
17. Houssay, B. A., and D. Potick: Compt. rend. Soc. de biol. **101:**940. 1929.
18. Lucke, H.: Arch. exp. Path. u. Pharmakol. **170:**166. 1933.
19. Cope, O., and H. P. Marks: J. Physiol. **83:**157. 1934.
20. Young, F. G.: J. Physiol. **87:**20. 1936.
21. Anselmino, K. J., and F. Hoffmann: Klin. Wchnschr. **12:**1245, 1435. 1933.
22. Russell, J. A., and L. L. Bennett: Proc. Soc. Exp. Biol. & Med. **34:**406. 1936.
23. Young, F. G.: J. Physiol. **90:**20. 1937.
24. Black, P. T., J. B. Collip and D. L. Thomson: J. Physiol. **82:**385. 1934.
25. Young, F. G.: Lancet, **2:**372. 1937.
26. Campbell, J., and C. H. Best: Am. J. Physiol. **123:**30. 1938.

THE SPECIFIC DYNAMIC ACTION TEST IN CASTRATED AND MENOPAUSAL WOMEN

WILLIAM M. MOFFAT

From the Research Department of the Santa Barbara Clinic and the Endocrine Clinic of the Santa Barbara General Hospital

SANTA BARBARA, CALIFORNIA

The specific dynamic action of protein may be defined as the total increase in heat production which follows the ingestion of food. As this varies with the amount and type of food eaten and may not be complete for approximately 14 hours, its determination as a routine clinical measure is obviously impractical. Consequently many investigators have tried various meals and determined the rise in heat production at arbitrarily chosen intervals with conflicting results. However, the authors of many books and articles in the current literature of the past 5 years have accepted as standard, and have advised the determination of the specific dynamic action, in suspected endocrinopathies, according to the method devised by Goldzieher and Gordon[1] and reported in 1933. The meal consists of the whites of 3 hard-boiled eggs, a thin slice of toast, and a cup of tea without sugar, cream or milk. The B.M.R. is determined before, and again 2 hours after, the meal. The term, specific dynamic action (S. D. A.) is used to express the difference between the readings. The results of their investigation disclosed an average S. D. A. or rise in the oxygen consumption of only 3.8 per cent in patients diagnosed as having hypofunction of the anterior lobe of the pituitary, as contrasted with an average rise of 16.3 in controls, 20.0 in hypo-ovarian or an-ovarian and 35.0 in pituitary tumors. If these results can be confirmed, the test is obviously of great value not only in the diagnosis of pituitary function but especially in the differential diagnosis between primary ovarian deficiency and ovarian deficiency secondary to hypofunction of the pituitary.

In this paper is presented an analysis of 150 such tests performed on 100 patients, 50 at the Santa Barbara General Hospital and 50 at the Santa Barbara Clinic, the two laboratories working independently. Sixty-one tests were made on 34 women with active menopausal symptoms. Twelve of the women were castrates and 22 were in the natural menopause. The results are compared with those obtained in the other 66 patients who served as controls.

In all cases, one or more basal metabolism estimations were made prior to the S. D. A. test in order to familiarize the patients with the procedure and to obtain a 'base line' of the fasting-oxygen consumption. When the S. D. A. test was made, the patient was kept in bed until after the final reading. Every patient had at least two readings at each time.

When the fasting metabolism on the day of the S. D. A. test was at too

[1] Goldzieher, M. A., and M. B. Gordon: Endocrinology 17:569. 1933.

great variance with the previously determined 'base line,' or when the result did not seem to fit the diagnosis the test was repeated on another day. Thus, most of the repeated tests were made on the castrated and menopausal women and for the reason that the first determined S. D. A. seemed too low. When this was done, the average of all the S. D. A. tests on the patient was used as the final figure. It is realized that this admits some tests which were not entirely satisfactory, but when the entire series is considered those findings thought to be too low were offset by others seemingly too high; and an important factor, namely, the natural tendency of an investigator to admit only the results suitable to his personal bias is eliminated.

RESULTS

The results of the investigation are condensed in a few tables. Table 1 shows the average S. D. A. in castrates, menopausal women and controls at

TABLE 1

No. of cases		Diagnosis	Average Specific Dynamic Action
S. B. Clinic	2	Castrates	2.5
	6	Menopause	5.2
	42	Controls	5.1
General Hospital	10	Castrates	3.6
	16	Menopause	5.0
	24	Controls	4.6
	100		

the Santa Barbara Clinic and at the General Hospital with the number of patients in each group. Fewer tests were performed on castrates and menopausal women at the Clinic because, until almost the end of the series, the cases were unselected, in that the tests were made to aid in the diagnosis of probable endocrinopathies; whereas later at the General Hospital the objective was the evaluation of the test itself, and consequently a large group of women with active symptoms of artificial or natural menopause was selected. As the results at the two laboratories were practically identical, the two groups are combined in the remaining tables. Thus table 2 shows in more condensed form that there

TABLE 2

No. of Cases	Diagnosis	Average Specific Dynamic Action
12	Castrates	3.4
22	Menopause	5.1
66	Controls	5.0
100		
	or	
34	All an-ovarian	4.5
66	Controls	5.0
100		

was no significant difference between the average S. D. A. in the an-ovarian women and in the controls.

In order to study the fluctuations in the S. D. A., the percentage of determinations of 10 and above is compared with the percentage below 10 in table 3. The spread is practically the same in the an-ovarians as in the controls. This

TABLE 3

	Specific Dynamic Action	
	Below 10	Above 10
Castrates	100%	0
Menopause	77%	23%
Controls	79%	21%
All an-ovarian	85%	15%

is seen again in table 4, which shows the percentage of patients with an S. D. A. of 0 or below.

Since there has been much discussion for many years as to whether or not a lowered S. D. A. is present in obesity, a summary of the results obtained in different states of nutrition is shown in table 5. Although this shows a high incidence of obesity among the an-ovarians, it fails to demonstrate that the obesity was a factor in lowering the S. D. A. On the contrary, it is seen that

TABLE 4

	Specific Dynamic Action=Zero
In castrates	17%
Menopause	18%
Controls	21%
All an-ovarian	18%

relatively more patients of normal weight had a lowered S. D. A. than did the overweight. In the case of the controls, the percentage of low results was approximately the same in the normal as in the overweight group.

COMMENTS

From the results tabulated above, it appears that, in these two laboratories, the S. D. A. test described is of questionable value in the diagnosis of ovarian deficiency. Now if the S. D. A. is the same in women with active menopausal symptoms as in other persons then the test is of little significance in the diagnosis of anterior pituitary function, since the evidence to date indicates that the symptoms of the natural menopause, or that following castration, are probably due to an overactivity of the anterior pituitary.

Included among the controls were 27 patients clinically considered as probably suffering from anterior pituitary deficiency, 7 as having primary ovarian deficiency, and 4 postmenopausal women without symptoms. None of these groups showed any significant variation in S. D. A. from the rest of the controls.

They are not summarized separately because there is always the possibility that a clinical diagnosis of anterior pituitary deficiency or of primary ovarian deficiency may be wrong, and in postmenopausal women without symptoms the pituitary is probably not overactive. But in the case of a castrated or menopausal woman who is having typical menopausal symptoms there is no question as to the diagnosis.

SUMMARY

An analysis is presented of 150 specific dynamic action tests performed on 100 patients, 50 at the Santa Barbara General Hospital and 50 at the Santa Barbara Clinic, the two laboratories having worked independently. The S. D. A. was determined 2 hours after a meal which consisted of the whites of 3 hard boiled eggs, a thin slice of toast and a cup of tea. Sixty-one tests were made in 34 women with active menopausal symptoms. Twelve of the women were castrates and 22 were in the natural menopause. The results are compared with those obtained in the other 66 patients who served as controls.

No significant difference between the two groups was found in either the average S. D. A. or the per cent of patients who showed a low S. D. A. The increased tendency toward obesity in the an-ovarian women is not a factor in lowering the S. D. A. Therefore the test is of doubtful value in the diagnosis of ovarian deficiency; and if menopausal symptoms are due to an overactivity of the anterior lobe of the pituitary, then this test is of doubtful value also as a measure of anterior pituitary function.

TABLE 5

Nutrition	No. Cases	% Total	Specific Dynamic Action		
			Below 5	Above 5	Total
In an-ovarian					
Overweight	24	70	42%	58%	100%
Normal	10	30	60%	40%	100%
Underweight	0	0	0%	0%	0%
Total	34	100			
In controls					
Overweight	29	44	48%	52%	100%
Normal	29	44	52%	48%	100%
Underweight	8	12	75%	25%	100%
Total	66	100			

THE INFLUENCE OF VARIOUS PHYSIOLOGICAL SUBSTANCES ON THE GLYCOGENOLYSIS OF SURVIVING RAT LIVER[1]

METHODS; INFLUENCE OF THE BILE SALTS

H. P. G. SECKEL
From the Department of Pediatrics, University of Chicago
CHICAGO, ILLINOIS

It is known that in children with von Gierke's glycogen storage disease (1), owing to an almost complete inhibition of the glycogenolytic enzyme, virtually no glycogenolysis occurs in the liver and other organs. In view of this fact it seems to be of interest to study the influence of various physiological substances, particularly of endocrine nature, on the glycogenolytic system of the normal liver. Among the hormones insulin, anterior pituitary and adrenal extracts were the first to be considered. Before this was done, a method of estimating glycogenolysis in surviving rat liver slices has been developed and the influence on the system of the conjugated bile salts, in which the writer was previously interested, has been tried experimentally. Thus, the present paper is in the nature of a methodical introduction to later communications concerned with the action of the various hormones.

In adults and grown-up experimental animals, Forsgren and his co-workers (2) have found that there exists a rhythmic cycle of the liver function during the 24-hour period of the day which, to a considerable extent, proved to be independent of the intake of food. A high bile-acid secretion and low glycogen storage was noted in the liver cells at certain times of the day, particularly in the late afternoon ('secretory phase') whereas the reverse proportion was stated at other times of the day, mostly in the early morning ('assimilatory phase'). Seckel and Kato (3) have shown that in nursing rats up to the age of 3 weeks the cyclic function of the liver develops only during the third week of life and was found to be virtually absent during the first two weeks. At the latter period, liver bile acids were demonstrated to be consistently high and liver glycogen to be consistently low throughout the entire day. This antagonism between bile acids and glycogen in the liver suggests the existence of some underlying relationship between these two physiological substances, especially an influence of the bile acids or their salts on the glycogen content of the liver cells.

In reviewing the literature, three different groups of experiments are found. In the first group, the effect on hepatic glycogen of a total or partial obstruction of the bile ducts and the subsequent accumulation of bile in the liver was studied in various animals. Under these conditions of artificial jaundice, liver glycogen was found to be considerably decreased (4, 5, 6, 7, 7a). The same was true of muscular glycogen (8). In dogs with complete biliary obstruction, Bollmann and Mann (9) observed that a diet rich in carbohydrates became increasingly necessary for the maintenance

[1] This work was supported by grants from the Douglas Smith Foundation for Medical Research and the Emergency Committee in Aid of Displaced Foreign Physicians.

of the lives of these animals. In the second group of experiments, extracts of organs and body fluids containing diastatic enzyme were mixed in vitro with a starch solution and amylolysis was estimated after varying intervals without and with addition of bile substances. The enzyme was shown to be activated by diluted whole bile and sodium glycocholate whereas the other bile salts proved to be inactive or to inhibit the diastase (10, 11, 12). With an improved method Willstätter and his coworkers (13) demonstrated that, by the addition of about 0.03 per cent sodium glycocholate, pancreatic anylolysis was inhibited at a pH of 6.8 and slightly activated at a pH of 8.0. In the third group of experiments, rabbits were starved from 4 to 6 days and then given, per os or parenterally, a certain amount of glucose without and with an additional injection of bile salts. The liver and other organs were analyzed for glycogen, lactic acid, etc., from 3 to 4 hours later. After the injection of small doses of sodium cholate or desoxycholate, Misaki (14) found that in the majority of injected animals the liver glycogen content was higher than in the non-injected controls. At higher doses, the opposite effect on liver glycogen was seen (15, 16). By determining lactic acid in the liver of rabbits, Tereoka (17) demonstrated a similar antagonistic effect of low and high doses of sodium cholate. These findings were corroborated by Tereoka by in vitro experiments performed with aseptic fresh ox-liver pulp kept in the thermostat at 37°C. for 48 hours with and without addition of 0.125 and 0.5 per cent sodium cholate.

METHODS

Lesser (18) has estimated in frogs what he called 'diastatic efficiency' of the liver. This was obtained by putting a whole liver lobe into 20 to 30 cc. of oxygenated Ringer's solution at 22°C. and determining its glycogen content after 4 hours as compared with that of another lobe immediately after death. Lesser's method was modified for warmblooded animals (rats) and the 'diastatic efficiency' of their liver tissue was studied by means of glycogen determinations. For this purpose, liver pieces were cut into fairly thin slices similar to those used in Warburg's experiments on tissue respiration (19) and placed for about 1 hour in a buffer solution at 37°C., with and without addition of various bile salts or other substances. A similar method was recently described by Willstätter and Rohdewald (20). The details of the technic are as follows.

Normal rats weighing from 150 to 350 gm. were fed on a stock diet rich in carbohydrate. Without previous fasting a rat was killed by a blow on the head between 9 and 11 A.M. and the liver removed as quickly as possible. Only one lobe of the liver was used for analysis (21); 0.4 to 0.8 gm. of it was immediately cut off and kept in a wet chamber. The rest of the liver lobe was then cut into 6 similar pieces, each varying in size from about 0.2 to 0.4 gm. After being weighed to milligrams, these pieces were cut successively with a razor blade into from 6 to 10 fairly thin slices and quantitatively transferred into six 50 cc. Erlenmeyer flasks containing 10 cc. of the following suspension solution: 8.0 gm. NaCl, 1.0 gm. $NaHCO_3$, 0.1 gm. KCl, 0.1 gm. $CaCl_2$, H_2O to 1000.0 cc. For each experiment, 90 cc. of this standard salt solution was mixed with 7.0 cc. of a M/15 Na_2HPO_4 solution and 3.0 cc. of a M/15 NaH_2PO_4 solution, the pH of this buffer being about 7.5 at 37°C. (22). Two flasks were used for controls containing bile-salt-free solutions, while 4 flasks were filled with buffer solutions containing two different concentrations of bile salts (duplicate determinations). The preparation of the liver slices took about 15 to 20 minutes. After that time the piece of liver, previously placed in a wet chamber, was

frozen in carbon dioxide ice. The exact moment of the freezing was taken as the starting or zero point of the experiment. At the same moment, the closed Erlenmeyer flasks containing the suspended liver slices were placed in the thermostat at 37°C. and then gently shaken from time to time. The frozen piece of liver, the 'zero' sample, was then cut into 2 parts, each of which was weighed and immediately thrown into and boiled for 15 minutes in a 30 per cent KOH solution (1 cc. to each 0.1 gm. of liver tissue) for glycogen determination. After 60, 90 or 120 minutes the liver slices were removed from the suspension solutions and likewise transferred into hot 30 per cent KOH solution. For glycogen determination Pflueger's method as modified by Good, Kramer and Somogyi (23) was used, consisting of precipitation by 95 per cent alcohol and centrifugation of the glycogen. After 2 hours hydrolysis with 0.6N hydrochloric acid the glucose was determined by Somogyi's modification of Shaffer and Hartmann's method (24). The result in milligrams of glucose was multiplied by 0.927 for glycogen estimation.

The glycogen figures obtained 15 to 20 minutes after death were found to vary between 2 and 8 gm. per 100 gm. of fresh liver (mostly between 3 and 5 gm. per cent). As to the duplicate determinations, the average deviation from the mean was found to be ± 1.83 per cent in 40 'zero' samples and ± 3.15 per cent in 113 preparations of suspended liver slices with and without bile salt. As a rule, the buffer solutions in which the liver slices had been suspended were measured and separately analyzed for dissolved glycogen. For this purpose Van Creveld's (25) method for determining glycogen in the blood was applied, using 5 cc. of the suspension solutions.

In a few experiments, an artificial system of chemically pure glycogen and a fresh glycogen-free rat liver pulp containing glycogenase was tested for glycogenolysis with and without addition of bile salt solutions. About 1.6 gm. of the pulp was suspended in 20 cc. of the above buffer solution and shaken for a short time. One cc. of this suspension was mixed in a test tube with 1 cc. of either bile-salt-free buffer or bile-salt solution. Then, 2 cc. of a 1 per cent glycogen solution was added and the test tubes were placed for 60 minutes in the thermostat at 37°C. For glycogen determination, the mixture was boiled with 4 cc. of 60 per cent KOH and the glycogen precipitated with 5 cc. of absolute alcohol.

Two preparations of conjugated bile salts obtained from fresh ox bile were used in these experiments: *a)* a preparation containing approximately 50 per cent sodium glycocholate and about 15 per cent sodium taurocholate, the rest being derivatives of desoxycholic acid and bile pigments; *b)* a highly purified preparation of about 60 per cent sodium glycocholate and about 40 per cent sodium taurocholate. Dissolved in concentrations of from 0.01 to 1.0 per cent in the above mentioned buffered salt solutions, the preparations showed the following properties. Whereas *a)* had a distinctly yellowish color, *b)* appeared as a virtually colorless solution. Both preparations were free of glycogen. The pH as estimated colorimetrically and with the quinhydrone electrode (at 35°C.) was found to rise by 0.2 at the most with rising concentrations of the bile salts. The surface activity of the bile-salt solutions was determined stalagmometrically, i.e., by the drop count method, y representing the number of drops per 3 cc. of bile-salt solution and z the number of drops per 3 cc. of bile-

salt-free buffer solution at 20°C. $\left(\text{drop count} = \frac{(100 \times y)}{z}\right)$. At bile salt concentrations of 0, 0.01, 0.05, 0.1, 0.5, and 1.0 per cent the following drop counts were found: preparation *a* 100, 113, 135, 153, 166, 171; preparation *b* 100, 113, 138, 153, 161, 168, respectively (18). This curve is the typical 'adsorption curve' characteristic of surface active substances. No glycogenase was found to be present in preparation *a*. A slight amount of the enzyme contained in preparation *b* was easily removed by placing the solution into boiling water for about 10 minutes.

EXPERIMENTAL RESULTS

Controls. Four experiments were made to check the influence on hepatic glycogenolysis of a change in pH of the buffer solution of from 7.3 to 7.7 such as might be caused by the addition of bile salts. Hodgson (26) working with pure glycogen and liver glycogenase found that an increase in pH of 0.25 caused an increase in glycogenolysis of about 5 per cent within 60 minutes. In the 4 experiments just referred to no definite change in glycogenolysis according to varying pH could be established. Thus, these experiments may be taken as bile-salt-free controls indicating the limits of error to be encountered in this type of experiment ($\pm$5 to 8 per cent). No controls concerning the salt ion factor of the bile salt solutions were deemed necessary since Willstätter (13) and Hodgson (26) have proved that the cation, i.e. sodium, does not influence the activity of the diastatic enzyme. The osmotic effect of the bile salts is virtually negligible, even in the higher concentrations used. The significance of the bile pigment factor will be discussed later.

Glycogenolysis Without Addition of Bile Salts. The results on glycogenolysis of surviving rat liver slices suspended in a bile-salt-free buffered salt solution are presented in part in table 1, columns 3 to 5. In 5 observations covering 80 to 120 minutes (90 minutes on the average) glycogenolysis amounted to an average of 29.7 mg. per gm. of liver, or 66.5 per cent of the 'zero' values. In 34 observations lasting for 60 minutes the respective figures were 19.4 mg. per gm., and 42.7 per cent (from 17.6 to 66.1 per cent). As in Noltie's (27) experiments, the rate of glycogenolysis does not depend upon the amount of glycogen present. One experiment was done with special reference to the time curve of rat liver glycogenolysis (*No. 36*). In unsliced pieces of rat liver, kept in Holmbergh's (28) buffer solution of pH 6.9 at 37°C., the 'zero' glycogen content 5 to 10 minutes after death was 69.05 mg. per gm.; after ½, 2, 3, 5½ and 24 hours the glycogen figures dropped to 40.80; 34.57; 29.45; 2.67 and 0 mg. per gm., respectively, which corresponds to a relative decrease in liver glycogen content of 41, 50, 57, 96 and 100 per cent (see fig. 1 of the following paper).

Glycogenolysis with Addition of Bile Salts. Thirty-six experiments on glycogenolysis of surviving rat liver slices under the influence of varying concentrations of bile-salt preparation *a* are presented in table 1 in the order in which they were actually done. Twenty-six experiments were made with preparation *b*. In the two last columns of table 1, the effect of the bile salt on glycogenolysis as compared to spontaneous bile salt-free glycogenolysis is cal-

culated in absolute and relative figures, the plus signs indicating inhibition of glycogenolysis, the minus signs increase in glycogenolysis (see figures 1 and 2, corresponding to preparations *a* and *b*). Under the influence of both bile-salt preparations, a biphasic curve of hepatic glycogenolysis, i.e. diastatic efficiency

TABLE 1. INFLUENCE OF VARYING CONCENTRATIONS OF BILE SALT PREPARATION *a* ON GLYCOGENOLYSIS OF SURVIVING RAT LIVER SLICES SUSPENDED FOR 60 MINUTES IN A BUFFERED SALT SOLUTION (pH 7.4 TO 7.5) AT 37°C.

No. of exper.	Concentr. of bile salt, %	Liver glycogen content					Difference in glycogenolysis between *A* and *B*	
		A. No bile salt			B. Bile salt			
		At start	60 min.	Diff.	60 min.	Diff.		
		mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	Per cent
43	0.10	55.24	19.36	−35.88	24.63	−30.61	+5.27	+14.7[1]
61[4]	0.40	42.67	15.13	−27.54	10.60	−32.07	−4.53	−16.5[2]
62[4]	0.25	57.13	20.10	−37.03	15.42	−41.71	−4.68	−12.6[3]
	0.50				12.10	−45.03	−8.00	−21.6
69	0.20	31.08	9.19	−21.89	10.34	−20.74	+1.15	+ 5.3[3]
	0.40				8.21	−22.78	−0.98	− 4.5
70	0.15	38.58	12.60	−25.98	15.63	−22.95	+3.03	+11.7[3]
	0.30				11.63	−26.95	−0.97	− 3.7
72	0.15	44.27	19.62	−24.65	25.89	−18.38	+6.27	+25.4
	0.30				21.93	−22.34	+2.31	+ 9.3
73[4]	0.10	40.86	17.10	−23.76	23.18	−17.68	+6.08	+25.5
	0.50				17.16	−23.70	+0.06	± 0
74[4]	0.05	54.05	28.72	−25.33	33.68	−20.37	+4.96	+19.6
	0.50				23.83	−30.22	−4.89	−19.3
75[4]	0.05	35.43	19.99	−15.44	21.16	−14.27	+1.17	+ 7.6
	0.50				15.42	−20.01	−4.57	−29.7
76	0.04	35.64	12.40	−23.24	17.54	−18.10	+5.14	+22.2
	0.40				12.25	−23.39	−0.15	± 0
77[4]	0.09	44.39	18.12	−26.27	28.45	−15.94	+10.33	+39.3
	0.45				19.87	−24.52	+1.75	+ 6.7
78	0.02	42.65	14.43	−28.22	18.20	−24.45	+3.77	+13.4
	0.20				21.50	−21.15	+7.07	+25.1
79[4]	0.07	52.40	27.28	−25.12	36.78	−15.62	+9.50	+37.8
	0.70				19.86	−32.54	−7.42	−29.6
80	0.01	44.07	17.78	−26.29	18.80	−25.27	+1.02	+ 3.9
	0.10				28.65	−15.42	+10.87	+41.3
90[4]	0.25	67.55	43.92	−23.63	42.03	−25.55	−1.89	− 8.0
	1.00				27.09	−40.46	−16.83	−71.3
91[4]	0.60	32.93	18.26	−14.67	13.62	−19.31	−4.64	−31.6
	0.80				12.08	−20.85	−6.18	−42.0
92	0.06	25.30	12.80	−12.50	15.96	− 9.61	+2.89	+23.1
	0.08				15.02	−10.28	+2.22	+17.8
94[4]	0.45	35.32	18.50	−16.82	15.27	−20.05	−3.23	−19.2
	0.90				11.23	−24.09	−7.27	−43.3
95[4]	0.03	41.76	25.30	−16.46	26.32	−15.44	+1.02	+ 6.2
	0.35				23.35	−18.41	−1.95	−11.8

[1] 120 min.
[2] 90 min.
[3] 80 min.
[1, 2, 3] Not buffered, pH about 8.0.
[4] Checked for glycogen dissolved in suspension solution.

or liver glycogenase action is shown by these experiments. At low bile-salt concentrations, there is a positive phase with the curve rising to from +10 to +40 per cent, at high bile-salt concentrations, there is a negative phase with the curve dropping to —50 per cent and more. Between these two phases, at medium bile-salt concentrations, there is a neutral zone with the figures falling within the limits of experimental error (±8 per cent). Comparing with one

another the curves obtained with preparations *a* and *b*, the negative figures seem to come less far down in preparation *b*. In general, it can be stated that in low concentrations (0.02 to 0.1 or 0.25 per cent) the conjugated bile salts considerably inhibit the action of the glycogenase of surviving rat liver slices whereas in high concentrations (0.25 or 0.4 to 1.0 per cent) the activity of the enzyme is strongly increased.

The suspension solutions were found to contain measurable amounts of

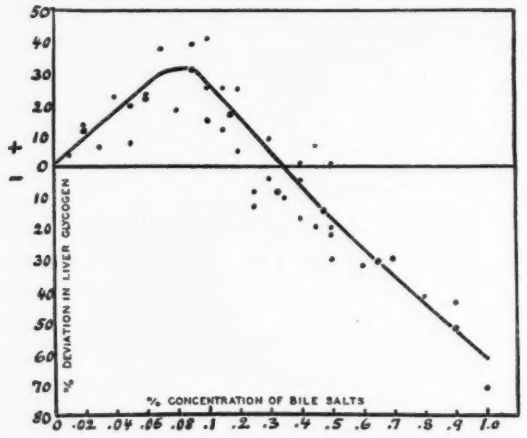


Fig. 1. Influence of various concentrations of bile-salt preparation *a* on glycogenolysis of surviving rat liver slices in percentage deviation from bile-salt-free glycogenolysis. The latter is represented by the zero x-line. Dots = individual experiments; circles = group averages connected by smoothed curve (see table 1).

dissolved glycogen only at higher concentrations of bile salts and roughly proportional to the concentrations. The following amounts of dissolved glycogen were determined (average figures in per cent of total glycogen present in the system): at 0.25 to 0.4 per cent bile salt, 10.5 per cent glycogen (14 determinations); at 0.45 to 0.6 per cent bile salt, 14.0 per cent glycogen (15 determinations); at 0.65 to 0.75 per cent bile salt, 16.1 per cent glycogen (8 determinations); at 0.8 to 1.0 per cent bile salt, 24.0 per cent glycogen (10 determinations, maximum figures from 27 to 32 per cent).

In two experiments testing the influence of bile-salt preparation *a* on an artificial system of pure glycogen and fresh liver glycogenase, glycogenolysis was found to be increased at all bile-salt concentrations, even those at which, in surviving liver slices, it is inhibited to a considerable extent. The 60 minute figures for glycogenolysis were: without bile salts, about 20 per cent; at 0.1, 0.5 and 1.0 per cent bile salts, 32.3, 37.7 and 43.0 per cent, respectively.

DISCUSSION

Various attempts have been made to explain how glycogen and glycogenase can exist together in the same cell and how, under these circumstances, glycogenolysis and glycogenesis are controlled in the liver. MacLeod (29) believes that by a turn to the acid side of the normal pH the previously inactive glycogenolytic enzyme is made active in the cell. However, this theory has been dis-

proved by Hodgson (26). Another hypothesis is traced back to the discoverer of glycogen, Claude Bernard. He assumed that, under certain circumstances, the glycogenase of the liver cell "does not come in suitable contact with the glycogen present in order to react with it" (quoted by Lesser, 30). Working independently, Bang (31), Lesser (30), and Lesser and Zipf (18) tried out this hypothesis experimentally. These investigators demonstrated an increase in glucose production, i.e. glycogenolysis by either placing frog livers in, or perfusing them with Ringer's solution and adding various concentrations of alcohols, ethyl ether, and chloroform. The lipid solvent, narcotic properties of these chemicals as well as their considerable surface activity were suggested as diminishing the supposed fixation of the diastatic enzyme to the intracellular

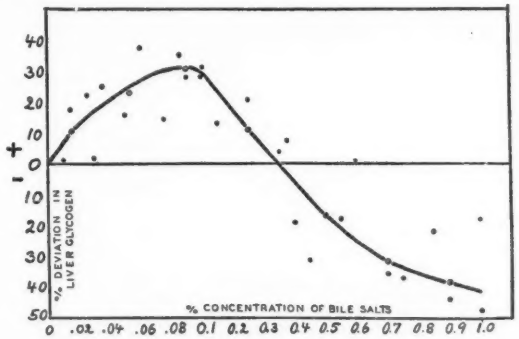


Fig. 2. Influence of various concentrations of bile-salt preparation *b*.

boundary surfaces. Recently, Willstätter and Rohdewald (20) have shown the hepatic diastase, among other enzymes, to be present in the cells in two different states, as an easily soluble 'lyo-enzyme' and a 'protoplasmatically bound desmo-enzyme'. On the other hand, liver glycogen was divided by them according to solubility into two portions called 'lyo-glycogen' and 'desmo-glycogen'. Hall and MacKay (32) histologically demonstrated the glycogen to be closely connected in the liver cell with the mitochondria of the protoplasm.

Bang, Lesser, and Willstätter did not take into consideration the most potent surface active substances physiologically occurring in the liver cells, namely, the bile acids or bile salts. In the light of their findings and hypotheses, the bile salt experiments presented in this paper might be interpreted as follows. The possibility of an increase in intracellular lyo-glycogen presented to the glycogenase at higher bile salt concentrations as suggested by the dissolution of glycogen from the liver slices is ruled out by the experiments done with the artificial glycogen-glycogenase system. More probably, in low concentrations the conjugated bile salts might partly inactivate the diastatic enzyme by either poisoning the lyo-glycogenase present in the cell or fixing it to the intracellular proteins, while, in higher concentrations the proportion of lyo- and desmo-glycogenase existing in the cell may be shifted to the more active lyo side, perhaps by damaging the cellular structure. In medium concentrations, both effects apparently neutralize each other. The great similarity of

the curves obtained with various concentrations of preparations *a* and *b* as to drop count and influence on glycogenolysis suggests an action by means of surface activity rather than different chemical constitutions. The bile pigments present only in preparation *a* apparently play no part in shaping the stalagmometric and glycogenolytic curves presented above (12).

Besides high blood sugar, oxygen supply, and a number of hormones, low concentrations of conjugated bile salts seem to be an important factor in stabilizing carbohydrate in the liver cells whereas high bile-salt concentrations, besides low blood sugar, asphyxia, and certain endocrine products, seem to be a means of mobilizing glycogen from the liver cells.

The experiments and discussions presented above somewhat clarify the earlier experiences of Wohlgemuth and the Japanese school previously reviewed. Furthermore, the daily cycle of the liver function as suggested by Forsgren (2) can be satisfactorily interpreted by these findings. The positive phase of the biphasic curves of bile salt glycogenolysis (fig. 1 and 2) seems to correspond to the 'assimilatory phase' of the liver cycle while the negative phase of those curves corresponds to the 'dissimilatory phase' of the liver. The latter phase was shown to be constantly present for the entire day during the first two weeks of a rat's life (3). The same holds true under the conditions of complete biliary obstruction, the glycogenase apparently being activated by the early accumulation of the bile salts. In clinical cases, occasionally the liver is so utterly incapable of storing glycogen from blood glucose that the glucose tolerance test shows abnormally high figures during the first hour (300 mg. per cent and more). This was recently seen in a baby, 3 months of age, with incomplete congenital obstruction of the bile ducts and intermittent jaundice.

Even in von Gierke's glycogen storage disease (1) the glycogenase, apparently abnormally fixed to the intracellular protein structures, seems to be activated in the liver and other organs during an intercurrent jaundice. This was evidenced in a case reported by Sundal (33) by a temporary decrease in size of the liver and a transient gain in weight of about 4 kg. over a 3-weeks period of a severe icterus. As a symptomatic treatment of the rare condition the feeding of high doses of a bile-salt preparation suggests itself under these circumstances. This therapeutic test was carried out by Linneweh of Berlin (34)[2] in the case of a 4-year-old boy. After feeding a bile-salt preparation for 3 months, the patient's liver was noticeably decreased, the abdominal circumference reduced by 6.5 cm., and the body weight increased by more than 1 kg. As experimental evidence for inaugurating this treatment, Linneweh, after reviewing part of the literature, reproduced one experiment done on a frog liver perfused with a buffered salt solution. After the addition of a certain amount of choleinic acid the glucose production of the liver as reflected in the perfusion solution was increased by nearly 100 per cent (7a).

SUMMARY

Lesser's method of estimating the 'diastatic efficiency' of frog liver lobes was modified for warm blooded animals by determining the 60 minutes de-

[2] This publication was received in Chicago in July 1937. At this time, the plan of the present paper was completely worked out and part of the experimental work had been done since April 1937.

crease in glycogen of rat liver slices surviving in a buffer solution at 37°C. The effect on this system of various concentrations of two bile-salt preparations containing different proportions of sodium glycocholate and taurocholate was studied in 62 experiments not including controls.

By expressing the 'bile-salt glycogenolysis' in terms of plus and minus deviations from 'bile-salt-free glycogenolysis' a biphasic curve of bile-salt glycogenolysis was obtained. The positive phase of this curve occurred at low concentrations of bile salts (0.01 to 0.2 per cent) while the negative phase was found at high concentrations (0.3 to 1.0 per cent). In the positive phase, the action of the liver glycogenase was inhibited by about 10 to 40 per cent, in the negative phase it was increased by about 10 to 70 per cent.

The physiological action of the surface-active bile salts on the intracellular fixation of the glycogenase to the protein boundary structures of the liver cell is briefly discussed.

The relationship of the experimental findings to Forsgren's daily cycle of the liver function, artificial and pathologic obstructive jaundice, and von Gierke's glycogen storage disease is considered. Symptomatic treatment of von Gierke's disease by feeding high doses of a bile-salt preparation may prove to be of value in suitable cases.

REFERENCES

1. von Gierke, E.: Beitr. z. path. Anat. u. z. allg. Path. **82**:497. 1929.
2. Forsgren, E.: Ztschr. Zellforsch. u. mikr. Anat. **6**:547. 1927. Ueber die Rhythmik d. Leberfunktion, des Stoffwechsel u. des Schlafes., Stockholm 1935.
3. Seckel, H., and K. Kato: Arch. Path. **25**:347. 1938.
4. Wickham-Legg, J.: Arch. exper. Path. u. Pharmakol. **2**:384. 1874.
5. Kuelz, E., and E. Frerichs: Arch. f. d. ges. Physiol. **13**:460. 1876.
6. Dastre, A., and M. Arthus: Arch. de Physiol. **1**:473. 1889.
7. Delhougne, F.: Arch. exper. Path. u. Pharmakol. **174**:88. 1934.

7a. Bernhard, F., Klin. Wchnschr. **8**:1789. 1939.

8. Hergenhahn, quoted after Wohlgemuth.
9. Bollmann, J. L., and Mann, F. C.: Ergebn. d. Physiol. **38**:445. 1936.
10. Wohlgemuth, J.: Biochem. Ztschr. **21**:447. 1909.
11. Buglia, G.: Biochem. Ztschr. **25**:239. 1910.
12. Minami, D.: Biochem. Ztschr. **39**:339. 1912.
13. Willstätter, R., E. Waldschmidt-Leitz and A. R. F. Hesse: Ztschr. f. physiol. Chem. **126**:143. 1923.
14. Misaki, K.: J. Biochem. **8**:235. 1927.
15. Kuramoto, T.: J. Biochem. **19**:315. 1934.
16. Fuziwara, K.: Biochem. Ztschr. **259**:203. 1933.
17. Tereoka, M.: Biochem. Ztschr. **249**:95. 1932.
18. Lesser, E. J., and K. Zipf: Biochem. Ztschr. **140**:439. 1923.
19. Warburg, O.: Der Stoffwechsel der Tumoren. Berlin 1926.
20. Willstätter, R., and M. Rohdewald: Ztschr. f. physiol. Chem. **225**:103. 1934; **203**:189. 1931; **229**:241, 255. 1934; Enzymologia **1**:213. 1936.
21. Lesser, E. J.: Biochem. Ztschr. **191**:175. 1927.
22. Clark, W. B.: The determination of hydrogen ions, Baltimore 1928.
23. Good, C. A., H. Kramer and M. Somogyi: J. Biol. Chem. **100**:485. 1933.
24. Somogyi, M.: J. Biol. Chem. **70**:599. 1926.
25. van Crefeld, S.: Arch. Dis. Childhood **9**:9. 1934.
26. Hodgson, Th. H.: Biochem. J. **30**:542. 1936.
27. Noltie, H. R.: Quart. J. Exper. Physiol. **24**:377. 1935.
28. Holmbergh, O.: Ztschr. f. physiol. Chem. **134**:68. 1924.
29. MacLeod, J. J. R.: Carbohydrate Metabolism and Insulin, London 1926.
30. Lesser, E. J.: Ergebn. d. inn. Med. u. Kinderh. **16**:279. 1919.
31. Bang, I.: Biochem. Ztschr. **49**:40. 1913.
32. Hall, E. M., and E. M. MacKay: Am. J. Path. **9**:205. 1933.
33. Sundal, A.: Acta Paediat. **19**:80. 1936.
34. Linneweh, F.: Monatschr. f. Kinderh. **70**:238. 1937.

THE INFLUENCE OF VARIOUS PHYSIOLOGICAL SUBSTANCES ON THE GLYCOGENOLYSIS OF SURVIVING RAT LIVER[1]

THE INFLUENCE OF INSULIN ADDED IN VITRO

H. P. G. SECKEL
From the Department of Pediatrics, University of Chicago
CHICAGO, ILLINOIS

Cammidge and Howard (1) have found that the activity of amylase and glycogenase extracted from the livers of dogs, cats and rabbits was inhibited at subnormal, though not at normal pH by the addition in vitro of small amounts of insulin. Davenport (2) was unable to reproduce this effect. When Hodgson (3) injected rabbits with insulin in sufficient doses to cause convulsions and then prepared a pure extract of glycogenase from the livers, he obtained only a small decrease of doubtful significance in the enzymatic action on a glycogen solution. Willstätter and Rohdewald (4) also got negative results by adding insulin to liver diastase solutions or dried liver pulp of various mammals. By preparing a fresh liver pulp containing diastase from glucose-fed mice, Scharles, Robb and Salter (5) succeeded in depressing glycogenase activity by injecting insulin 1 to 4 hours before death. In vitro effects of a similar nature reported by Partos (6) had been obtained by an unsatisfactory method.

A few papers have been published concerning the influence of the pancreatic hormone on the diastatic efficiency of surviving liver tissue. Grafe, Reinwein and Singer (7) studied the problem on liver slices of guinea pigs, rabbits and dogs using the Warburg apparatus and determining the glucose dissolved into the suspension solution by post-mortem glycogenolysis. In three experiments, the insulin was added directly in vitro and found to inhibit the 60 minute glycogenolysis by an average of about 40 per cent. Because the results thus obtained were inconsistent, liver pieces were removed for slicing from anesthetized animals before and 1 to 2 hours after the injection of insulin. Again, insulin produced a distinct inhibition of glycogenolysis within 60 minutes. The same effect was observed by Siegel (8) at shorter intervals in unsliced surviving livers of mice. The in vitro effect of crystallized insulin on the glycogenolysis of liver slices prepared from pigs, calves, dogs and cats was investigated by Willstätter and Rohdewald (4). In most of the experiments, soluble starch was added to the suspension solutions causing a slowing down of the diastatic process. Under these circumstances, amylolysis was found to be accelerated by insulin when the enzyme was inactive, as early after death, and to be inhibited when it was active, as for instance after the liver had 'grown old' for 40 to 60 minutes. When no starch was added to the solutions glycogenolysis proceeded at a high rate and was inhibited by insulin (28 and 43 per cent in 1 and 2 hours, respectively).

[1] This work was supported by grants from the Douglas Smith Foundation for Medical Research and the Emergency Committee in Aid of Displaced Foreign Physicians.

The experiments reported in this paper are concerned with the influence of insulin, added in vitro, on the glycogenolysis of surviving rat liver slices. No starch was added to the suspension solution. Glycogenolysis was estimated by determining directly the glycogen content of the liver slices. In most details, the methods applied were the same as described in the first communication (9).

METHODS

Most of the rats used in this series of experiments were non-fasted males 2 to 5 months old (February to June, 9 to 11 A.M.). The relation between the amount of liver tissue and the volume of the suspension solution was about 0.3 gm. to 10 cc., occasionally 0.2 gm. to 5 cc. This is a little less than the 1.5 gm. of liver suspended in 37 cc. of solution by Willstätter and Rohdewald (4). As a rule, 8 Erlenmeyer flasks rather than 6 were run in one experiment yielding 4 duplicate determinations of liver glycogen. (Methods by Good, Kramer and Somogyi, 1933; and Shaffer, Hartmann and Somogyi 1926.) The starting or 'zero' points of the experiments were thereby postponed to between 20 and 28 minutes after the animal's death. The closed gas atmosphere and continuous shaking of the Warburg technic could apparently be dispensed with since no gas pressures were measured and the shaking does not produce any different results (4). Instead of the original Warburg system a phosphate buffer was mixed with the salt solution—the gas room being air—such as to maintain a pH of about 7.4 at 37°C. For further details of the technic see the preceding communication (9).

Insulin Dosage. As far as can be computed from the data of Grafe and his collaborators (7), the 0.02 U of insulin added to each cc. of suspension solution equaled about 6 U per 1 gm. of fresh liver. Willstätter and Rohdewald (4) added 92 U per 1.5 gm. (= 60 U per 1 gm.) of liver tissue suspended in 37 cc. of solution which amounts to about 2.5 U per cc. In a few experiments, 1.6 and 3.2 U per cc. were used. The dosage applied by Bach and Holmes (10) for glyconeogenesis studies was of the same order of magnitude (0.15 to 4 U per cc.) although the amount of tissue prepared was considerably smaller. In his work on insulin and tissue respiration Laser (11) also had added 0.04 to 4 U per cc. of suspension solution. In the following experiments, 0.3 to 6.4 U of insulin per cc. of solution was used which roughly corresponds to 10 to 210 U per 1 gm. of liver tissue. The hormone used was the commercial solution of insulin (Lilly) in concentrations of either 40 or 80 U per cc. of distilled water.

EXPERIMENTAL RESULTS

Controls. The pH of the suspension solutions was determined colorimetrically and found to be changed by not more than —0.2 by the addition of insulin. Such a change in pH does not influence the rate of liver glycogenolysis as was shown in the foregoing communication. Further controls were carried out by adding to the buffered salt solutions distilled water in amounts corresponding to those of the insulin preparations used. Up to 1 cc., in 3 experiments the water did not cause any change in glycogenolysis outside the limits of experimental error (±8 per cent). In two experiments using 1.4 cc. of water, an inhibition of about +14 per cent was obtained. No experiments using more than 1.2 cc. of the watery insulin solution were therefore recorded.

TABLE 1. INFLUENCE OF VARYING CONCENTRATIONS OF INSULIN ADDED IN VITRO ON THE GLYCOGENOLYSIS OF SURVIVING RAT LIVER SLICES SUSPENDED FOR 60 MINUTES IN A BUFFERED SALT SOLUTION (pH 7.4, T. 37°C.).

No. of exper.	Concentr. of insulin u/cc.	Liver glycogen content						
		A. No insulin			B. Insulin		Difference in glycogenolysis between *A* and *B*	
		At start mg./gm.	60 min. mg./gm.	Diff. mg./gm.	60 min. mg./gm.	Diff. mg./gm.	mg./gm.	Per cent
130	0.3	41.96	28.80	−13.16	28.62	−13.34	−0.18	± 0
	0.8				29.00	−12.96	+0.20	± 0
131	2.0	46.57	34.19	−12.38	37.20	− 9.37	+3.01	+24.3
	4.0				39.53	− 7.04	+5.34	+43.1
132	1.6	42.07	23.91	−18.16	27.46	−14.61	+3.55	+19.5
	3.2				30.89	−11.18	+6.98	+38.4
133	2.6	43.16	22.78	−20.38	27.12	−16.04	+4.34	+21.2
	2.6[1]	43.16	26.40	−16.76	30.03	−13.13	+3.63	+21.6
134	4.0	51.20	25.18	−26.02	31.04	−20.16	+5.86	+22.5
	4.0[2]	51.20	31.76	−19.44	33.18	−18.02	+1.42	+ 7.4
138	4.0	43.09	20.62	−22.47	25.11	−17.98	+4.49	+20.0
146	0.8	49.98	37.22	−12.76	38.90	−11.08	+1.68	+13.1
147	3.2	47.82	32.91	−14.91	38.72	− 9.10	+5.81	+39.0
148	3.2	40.46	21.00	−19.46	24.08	−16.38	+3.08	+15.8
149	2.8	31.80	16.16	−15.64	20.01	−11.79	+3.85	+24.7
150	2.4	49.05	28.76	−20.29	32.20	−16.85	+3.44	+16.9
	4.8				31.70	−17.35	+2.94	+14.5
151	1.2	54.80	38.22	−16.58	39.78	−15.02	+1.56	+ 9.4
	3.6				39.43	−15.37	+1.21	+ 7.3
153	2.0	39.51	21.70	−17.81	27.55	−11.96	+5.85	+32.9
	3.6				28.66	−10.85	+6.96	+39.1
	4.4				27.42	−12.09	+5.72	+32.1
155	2.4	39.41	17.34	−22.07	20.91	−18.50	+3.57	+16.1
	4.8				21.77	−17.64	+4.43	+20.0
157	4.0	52.27	25.50	−26.77	32.26	−20.01	+6.76	+25.2
	4.0[3]				31.20	−21.07	+5.70	+21.3
158	3.6	45.31	27.37	−17.94	31.12	−14.19	+3.75	+21.0
	3.6[4]				34.04	−11.27	+6.67	+37.3
159	*2.4*	54.00	30.37	−23.63	35.05	−18.95	+4.68	+20.0
	4.8				36.35	−17.65	+5.98	+25.4
161	*2.0*	43.46	22.59	−20.87	27.10	−16.36	+4.51	+21.6
	3.2				30.07	−13.39	+7.48	+35.8
	4.0				31.20	−12.26	+8.61	+41.2
162	*2.8*	52.02	27.55	−24.47	31.27	−20.75	+3.72	+15.2
	4.4				32.53	−19.49	+4.98	+20.3
	5.2				31.66	−20.36	+4.11	+16.8
164	*1.2*	42.67	22.03	−20.64	26.48	−16.19	+4.45	+21.6
	6.4				26.91	−15.76	+4.88	+23.7
165	*2.8*	61.98	30.56	−31.42	36.86	−25.12	+6.30	+20.1
166	*4.4*	51.49	21.98	−29.51	30.03	−21.46	+8.05	+27.3
	4.4[5]	51.49	6.01	−45.48	7.46	−44.03	+1.45	+ 3.2
167	0.4	38.33	16.41	−21.92	17.40	−20.93	+0.99	+ 4.5
	1.6				19.61	−18.72	+3.20	+14.6
	5.6				20.76	−17.57	+4.35	+20.0
168	*4.4*	46.97	25.69	−21.28	29.31	−17.66	+3.62	+17.0
	4.4[6]	46.97	11.98	−34.99	12.95	−34.02	+0.97	+ 4.5
169	*2.0*	39.10	16.56	−22.54	19.82	−19.28	+3.26	+14.5
	4.8				18.71	−20.39	+2.15	+ 9.6
170	*2.8*	61.40	35.41	−25.99	37.28	−24.12	+1.87	+ 7.2
	5.2				37.63	−23.77	+2.22	+ 8.5
	6.0				39.75	−21.65	+4.34	+16.7

[1] 0.5 per cent glucose in solution.
[2] 0.1 per cent bile salt in solution.
[3] Insulin heated at 100°C. for 10 minutes.
[4] Italicized u taken from insulin 'u 80.'
[5] 2 per cent bile salt in solution.
[6] 1 per cent bile salt in solution.—No. 154, 155, 157, 158, 159 were run with distilled water controls (0.6 to 1.4 cc.).

As to the duration of the insulin experiments 60 minutes was found to be the most suitable time. In a few samples taken at random the suspension solutions were found to be practically free of glycogen at the end of the insulin experiments.

'Zero' Glycogen Figures. The values obtained 20 to 28 minutes after the animal's death were between 3.1 and 6.2 gm. per cent of fresh liver. The average deviation from the mean of the duplicate determinations was ± 1.95 per cent in 29 'zero' samples and ± 2.86 per cent in 90 sliced samples suspended for 60 minutes without and with addition of insulin.

Glycogenolysis Without Addition of Insulin. In a number of experiments

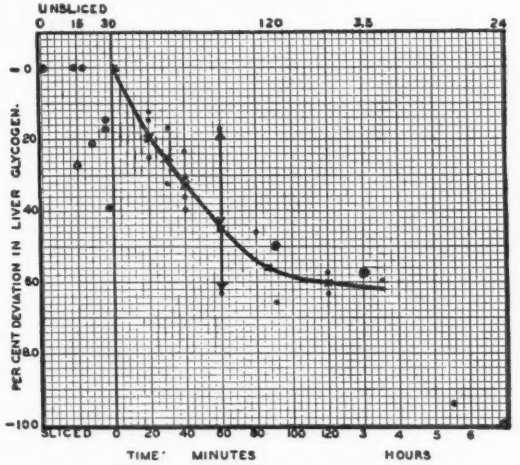


Fig. 1. Spontaneous glycogenolysis of surviving rat livers in percentage deviation from zero glycogen figures. *a)* Unsliced liver pieces, zero glycogen taken 3 minutes after death: circles = individual livers, kept in wet chambers at room temperature; dotted circles = single liver (*No. 36*) placed in Holmberg's solution at 37°C. *b)* Sliced liver pieces, zero glycogen taken 20 to 28 minutes after death: dots = individual livers, suspended at 37°C.; crosses = group averages of same, connected by smoothed curve. At 60 minutes, the two crosses represent averages of 34 and 43 determinations, resp., the maximum swing being indicated by the arrows.

(fig. 1) liver glycogen was determined immediately after the animal's death and 15 to 26 minutes later, the unsliced liver pieces being preserved at 20°C. in a wet chamber. In two cases, no difference was found within 15 and 16 minutes, respectively, whereas in the majority of experiments a decrease in liver glycogen of from 15.2 to 27.4 per cent took place in 15 to 26 minutes. This is in agreement with the observations of Willstätter and Rohdewald (4) who found that, shortly after death, the diastatic enzyme may be encountered in physiological conditions varying between complete inhibition and high grade activity. Therefore, Evans, Tsai and Poung's (12) finding of a 25 to 40 per cent disappearance of glycogen from roughly sliced cat's liver within the first 8 minutes after death should not be generalized for other mammalian species. The course of glycogenolysis of unsliced rat liver pieces covering the 24 hour period at 37°C. is presented in fig. 1 (*No. 36*).

In the same graph, figures are given for glycogenolysis of rat liver slices suspended in buffered salt solutions for 20 minutes to 5½ hours. There is a gradual decrease in liver glycogen content to nearly zero within this time proceeding most rapidly in the beginning of the curve. The largest number of experiments was done at 60 minutes. In 77 determinations, an average decrease in liver glycogen of about 44 per cent was seen during this time, the swing being between about 20 and 60 per cent. Here, too, the wide variation in the physiological activity of the glycogenolytic enzyme is to be observed. The

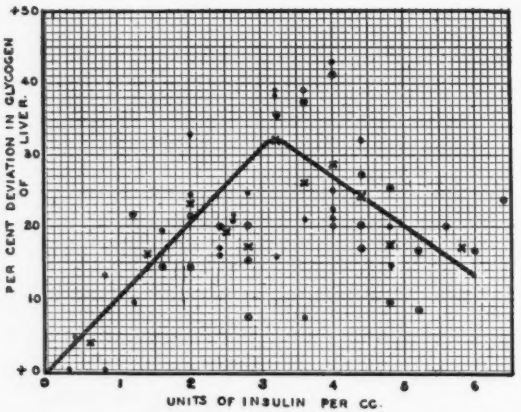


Fig. 2. Inhibition by insulin of glycogenolysis of surviving rat liver slices in percentage deviation from insulin-free glycogenolysis. The latter is represented by the zero x-line. Dots = experiments with insulin (U 40); circles = experiments with insulin (U 80); crosses = group averages conected by smoothed curve (see table 1).

figures presented in this series hold true only for the use of phosphate buffer solutions; in Ringer's solution glycogenolysis proceeds at a slower rate.

Glycogenolysis with Insulin Added in Vitro. The results of 51 determinations are listed in table 1 in the order in which they were actually done (cf. fig. 2). The zero x-line of fig. 2 represents the insulin-free, spontaneous glycogenolysis of each individual experiment, the plus values indicating inhibition of glycogenolysis in per cent of spontaneous glycogenolysis. Almost all figures obtained with addition of insulin show an inhibition of glycogenolysis which, up to a concentration of 4 U per cc., is roughly proportional to the insulin concentration. At 0.3 to 0.8 U per cc., the effect is within the limits of experimental error. The peak of the curve representing inhibitions of glycogenolysis of from 30 to 40 per cent coincides with concentrations of 3.2 to 4 U per cc. of insulin. The highest individual inhibition is 43.1 per cent at 4 U per cc. At concentrations higher than 4 U per cc., the curve of insulin inhibition of glycogenolysis again drops to lower percentages. The considerable variation in degree of inhibition at the same insulin concentration in different experiments may be attributed to a peculiar property of the individual liver preparation rather than to such differences as sex, age, original liver glycogen content, or primary activity of the glycogenolytic process (4).

Three experiments were done using bile-salt preparation *a* of the preceding communication (9) in addition to insulin. The inhibition of glycogenolysis caused by a 0.1 per cent bile-salt concentration was not increased to any appreciable degree by the addition of 4 U per cc. of insulin. On the other hand, the acceleration of glycogenolysis caused by 1 and 2 per cent bile-salt solutions was not at all inhibited by adding 4.4 U per cc. of insulin. Heating the insulin at 100°C. for 10 minutes does not remove the characteristic effect (13).

DISCUSSION

If it may be assumed that the differences in glycogenolysis of surviving rat liver slices as observed with and without addition of insulin bear any relationship to what is going on in the living animal, it may be stated that the primary action of insulin on the liver is the inhibition of glycogenolysis. This antidiastatic effect of insulin seems to be reproduceable only in the living or at least the surviving liver tissue and not with a pure extract of the diastatic liver enzyme (1 to 6). According to Willstätter and Rohdewald (4) inhibition of liver glycogenolysis by insulin is due to its combination with the intracellular activators of the glycogenase (peptids, amino-acids, etc.) thus giving preponderance to the inhibitors of the enzyme (proteins, amines, etc.). In another view, insulin furthers re-adsorption of the glycogenase to intracellular surface structures (14, 15, 16, 17).

A similar interpretation of the primary action of insulin on the liver has been advanced by several writers who worked with the intact living animal (14, 15, 18, 19). The only species in which insulin injections produce an accumulation of glycogen in the liver is the young rabbit (20, 21). In all other species of experimental mammals, liver glycogen is mobilized by insulin injections, most probably as an indirect or secondary reaction to hypoglycemia, adrenalin secretion, and other hormonal effects. This "implies that the internal adjustment in the organism makes the intact animal unsuitable for the investigation of the essential action of hormones" (20). Nevertheless, insulin can be demonstrated to inhibit liver glycogenolysis in the living animal, too, "especially when this process proceeds at an augmented rate" (15). This is the case in urethane and ether anesthesia, early after epinephrine injection, and in pancreatic and phlorizin diabetes (14, 22, 23, 24). Glycogenolysis, also, is speeded up in the normal animal's liver some time after death, and again the hepatic glycogenase is inhibited under the influence of insulin.

As to human pathology, Popper and his collaborators (25) observed a considerable post-mortem inhibition of the diastatic efficiency of the liver for its own glycogen in insulin treated diabetics and in a case of a dehydrated 6-months-old baby who died after a severe insulin shock. In the rare cases of a tumor of the pancreatic islands with an overproduction of insulin and attacks of hypoglycemia, Wilder (26) and Cragg (27) and their coworkers have demonstrated large amounts of liver glycogen difficult to be mobilized by adrenaline injections. Since the writer had an opportunity to investigate for himself tissue glycogen and glycogenolysis in a similar case, this subject will be discussed later. Also, the relationship of this study to von Gierke's glycogen storage disease is to be considered in a later communication.

SUMMARY

With a method described in an earlier publication, rat liver glycogenolysis as it normally occurs in surviving tissue slices suspended in a buffered salt solution, was shown to be inhibited to a considerable extent by large doses of insulin added in vitro (up to 43 per cent at 4 U per cc.).

Because of this finding and the results reported in the experimental and clinical literature, the essential action of insulin on the liver is believed to be an inhibition of the glycogenolytic process particularly when the latter is proceeding at a high rate.

REFERENCES

1. Cammidge, P. J., and H. A. H. Howard: J. Metabol. Research **5**:95. 1924.
2. Davenport, H. A.: J. Biol. Chem. **70**:625. 1926.
3. Hodgson, T. H.: Biochem. J. **30**:542. 1936.
4. Willstätter, R., and M. Rohdewald: Enzymologia **1**:213. 1936.
5. Scharles, F. H., P. D. Robb and W. T. Salter: Am. J. Physiol. **111**:130. 1935.
6. Partos, A.: Fermentforsch. **10**:50. 1928.
7. Grafe, E., H. Reinwein and H. Singer: Arch. f. exper. Path. u. Pharmakol. **119**:91. 1926.
8. Siegel, R.: Klin. Wchnschr. **8**:1069. 1929.
9. Seckel, H. P. G.: Endocrinology **23**:751. 1938.
10. Bach, S. J., and E. G. Holmes: Biochem. J. **31**:89. 1937.
11. Laser, H.: Biochem. Ztschr. **241**:36. 1931.
12. Evans, C. L., C. Tsai and F. G. Young: J. Physiol. **73**:67. 1931.
13. Krogh, A., and A. M. Hemmingsen: Biochem. J. **22**:1231. 1928.
14. Macleod, J. J. R.: Insulin. Part I, Physiology. Baltimore 1925. The fuel of life. Princeton 1928. Lancet **217**(2), I; 55; 107. 1929.
15. Cori, C. F.: Physiol. Rev. **11**:143. 1931.
16. Przylecki, St. J.: Erg. Enzymforsch. **4**:11. 1935.
17. Lesser, E. J., and K. Zipf: Biochem. Ztschr. **140**:439. 1923.
18. Geelmuyden, H. C.: Ergebn. d. Physiol. **30**:1. 1930.
19. Long, G. N. H., and A. White: Ergebn. d. Physiol. **40**:164. 1938.
20. Goldblatt, M. W.: Biochem. J. **23**:83. 1929; **24**:1199. 1930; J. Physiol. **79**:286. 1933.
21. Corkill, B.: Biochem. J. **24**:779. 1930.
22. Macleod, J. J. R., E. C. Noble and M. K. O'Brien: Tr. Roy. Soc. Canada **18**:129, Sec. V. 1924.
23. Sahyun, M., and J. Luck: J. Biol. Chem. **85**:1. 1929.
24. Cori, G. T., C. F. Cori and K. W. Buchwald: J. Biol. Chem. **86**:375. 1930.
25. Popper, H., and O. Wozasek: Virchow's Arch. f. Path. Anat. **288**:673. 1933; Ztschr. f. d. ges. exper. Med. **77**:414. 1931; **83**:682. 1932.
26. Wilder, R. M., F. N. Allan, M. H. Power and H. W. Robertson: J.A.M.A. **89**:348. 1927.
27. Cragg, R. W., Power, M. H., and Linden, M. C.: Arch. Int. Med. **60**:88. 1937.

INSULIN THERAPY IN MENTAL DISEASES

D. V. CONWELL AND C. J. KURTH
From the Department of Neuropsychiatry, Hertzler Clinic
HALSTEAD, KANSAS

On using Sakel's (1) method of insulin therapy on patients with schizophrenia and other functional types of mental diseases, we were confronted by exigent problems. Some of these were the control of the reactions to prevent immediate disaster, the observing of the early changes that might occur, especially in the metabolic processes, and the persistent search for similar late changes. We were particularly interested in seeing whether or not this rather drastic disruption of the endocrine mechanism might produce undesirable changes in the endocrine system. The mental diseases requiring treatment were serious enough to warrant our taking reasonable chances.

In December 1936 we began using insulin shock therapy in schizophrenia. Since then we have so treated 37 patients with schizophrenia, 9 with manic depressive psychosis, 6 with melancholia and 2 patients with psychasthenia. Nine of these patients had an associated thyrotoxicosis. The age range for the entire group was from 17 to 75 years. The average age of the schizophrenia patients was 24 years for the simple type, 27 years for the hebephrenic type, 25 years for the catatonic type and 44.5 years for the paranoid type. The average age of the patients with manic depressive psychosis was 44.5 years, of the melancholia patients was 62 years and of the psychasthenia patients was 46.5 years. The average age for the entire group was 36.9 years. Of the entire group 37 were females and 17 were males. Twenty-six of these patients were housewives, 11 were students or teachers, 10 were professional men and women and 7 were farmers. Seventeen patients had had previous psychotic episodes; 12 of these were of the schizophrenic group, 3 of the manic depressive group and 2 of the melancholia group. The remainder were seen in their first mental breakdown. The current psychosis range of the entire group was from 2 weeks to 5 years. Thirty-five had been ill for 6 months or less, 5 from 6 to 12 months and 14 for more than a year.

The preliminary study included urinalysis, blood count, blood-Wassermann reaction, venous blood-calcium and phosphorus determinations, fasting capillary blood-sugar readings, sugar-tolerance curve, spinal fluid analysis for pressure, pressure changes, cells, globulin and Wassermann reaction, basal metabolic rate and roentgenograms of a flat and a long bone. In the first half of the series there were no early or late variations from the normal in the blood-calcium or phosphorus values and no bone changes, so these observations were discontinued. The blood-Wassermann reactions and spinal fluid findings were uniformly normal. Reasonably accurate basal metabolic rates

were obtained on 5 patients but the cooperation of the other patients was so poor that it was impossible to obtain this data. Eighty-five per cent of the patients had a mild secondary anemia. We were unable to observe blood-count changes of importance. A slight leucocytosis was frequently encountered, especially during a period of dehydration.

The sugar-tolerance curves or the fasting capillary blood-sugars have been of definite value to us in the pre-treatment concept of the patients' insulin tolerance. The sugar-tolerance curves obtained were high in 24, average or normal in 7 and flat in 5 patients. The fasting capillary blood-sugars on the remaining 18 patients showed 2 to be high, 8 normal and 8 low. The fasting blood-sugars used as checks on the sugar-tolerance curves usually agreed with the curve findings. The sugar-tolerance curve or the fasting capillary blood-sugar was high in 75 per cent, average in 12½ per cent and low in 12½ per cent of the patients with the simple type of schizophernia (table 1). It was high in 50 per cent, normal in 25 per cent and flat in 25 per cent of the hebephrenic type (table 2); high in 55 per cent and normal in 45 per cent of the paranoid type (table 3); and high in 83 per cent and flat in 17 per cent of the catatonic type (table 4). It was high in 56 per cent, average in 22 per cent and flat in 22 per cent of the patients with manic depressive psychosis (table 5); high in 66 per cent, average in 17 per cent and flat in 17 per cent of the patients with melancholia (table 6); and high in 50 per cent and average in 50 per cent of the patients with psychasthenia (table 6). In order that we might have some measure for comparison we considered a peak dose of less than 40 U of insulin as a low tolerance, of 40 to 55 U as average and above 55 U as a high tolerance. With 8 exceptions, the patients with high blood-sugar values were able to tolerate large doses of insulin. One of the patients with average sugar values had a high insulin tolerance, 6 of them had a low insulin tolerance and the remainder of the average group had the anticipated tolerance range of 40 to 55 U of insulin. Two-thirds of the patients with low sugar values had relatively high insulin tolerance and the remainder had to be treated with great caution as they had severe shocks on small doses of insulin. As a rule the patients with low or average blood-sugar values built up their insulin tolerance very slowly. Because we felt that the fasting blood-sugars and sugar-tolerance curves were of value as a preliminary guide in the therapy, we began to get daily blood-sugar determinations on these patients 5 hours after the insulin was given.

In 1923 Cowie, Parsons and Raphael (2) reported the use of insulin in tonic doses to improve the appetite and general physical condition of mental patients Since then insulin has been used in the same way according to the reports of many authors. Such use of insulin was not followed by improvement of the mental state of enough patients to make this method of value. Considering this point, we did not feel that improvement of the appetite and the general physical condition was of primary importance. It did not seem that the ingestion of carbohydrates would do other than neutralize the insulin. Since we were not desiring a maintenance or increase of the glycogen reserve, we controlled the diet, as it did not seem logical to increase the insulin dosage

TABLE 1. SCHIZOPHRENIA, SIMPLE TYPE

Case	Age	Hosp., days	Treatment, days	Maximum dose u	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin u used
					1	2	3				
1	25	50	28	35	123	348	162	Occup.	118	4	450
2	36	114	27	10				Occup.	122	5	940
3	25	36	31	30	300	155	109	Occup.	110	5	485
4	17	108	95	130				Occup.	114	12	4990
5	31	107	103	60	107	254	119	None	96	16	3560
6	19	40	30	45	198	190	130	Occup.	123	4	797
7	22	20	7	15	106	213	105	Occup.	106	1	80
8	25	92	55	35	102	154	106	None	104	9	1120

In all of the tables the sugar values are expressed in milligrams per 100 cc. of capillary blood.

TABLE 2. SCHIZOPHRENIA, HEBEPHRENIC TYPE

Case	Age	Hosp., days	Treatment, days	Maximum dose u	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin u used
					1	2	3				
1	28	125	116	150	311	254	190	Social	123	11	5910
2	27	43	37	80	336	311	158	Occup.	128	6	1450
3	28	63	42	150	336	198	133	Occup.	124	7	3390
4	18	88	77	65	263	198	151	Occup.	118	11	3055
5	17	185	139	85	249	123	58	Social	77	14	4625
6	30	102	88	55	71	365	292	Social	76	13	3200
7	20	66	57	100				Occup.	122	9	2295
8	29	163	130	45	198	141	107	None	105	22	3510
9	27	65	54	50	196	137	105	Occup.	103	8	1460
10	27	72	42	50	194	128	86	Occup.	77	7	1420
11	35	137	117	40	155	110	101	Occup.	77	18	2895
12	38	155	121	50	134	102	88	None	92	20	2325

TABLE 3. SCHIZOPHRENIA, PARANOID TYPE

Case	Age	Hosp., days	Treatment, days	Maximum dose u	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin u used
					1	2	3				
1	37	77	56	90	290	236	112	Occup.	118	8	3625
2	44	114	53	40	249	151	112	Occup.	106	7	1080
3	64	80	75	40	117	271	246	Occup.	114	8	910
4	55	88	51	25				Occup.	122	4	1105
5	50	71	57	60	223	198	123	Social	119	8	1915
6	42	119	33	55	228	155	122	None	110	5	1015
7	33	39	28	55				Occup.	100	4	860
8	50	83	53	30				Occup.	104	4	425
9	33	32	11	15				Occup.	100	2	150
10	54	59	31	30				Occup.	96	5	585
11	48	89	59	25				None	93	5	1205

TABLE 4. SCHIZOPHRENIA, CATATONIC TYPE

Case	Age	Hosp., days	Treatment, days	Maximum dose U	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin U used
					1	2	3				
1	19	172	144	130	300	280	136	None	125	17	9225
2	18	96	75	100	315	212	122	None	118	9	3105
3	23	93	73	90	324	203	117	Occup.	109	8	2710
4	38	73	70	30	100	311	203	Social	100	6	595
5	32	69	59	50				Occup.	120	4	1230
6	31	141	116	90	145	125	111	Social	92	12	4250

TABLE 5. MANIC DEPRESSIVE PSYCHOSIS

Case	Age	Hosp., days	Treatment, days	Maximum dose U	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin U used
					1	2	3				
1	51	54	49	55	109	324	280	Social	109	11	1300
2	43	100	94	80	118	300	203	Social	116	12	2025
3	56	69	50	45	280	249	140	Occup.	120	6	1270
4	38	131	123	70				None	123	16	5115
5	19	94	84	90				Occup.	116	20	4265
6	38	192	174	60	249	168	101	Social	90	32	5710
7	29	86	72	55				Social	101	9	1405
8	33	21	14	75	165	140	106	Occup.	104	2	920
9	51	27	25	30	69	168	133	Occup.	77	8	290

TABLE 6. MELANCHOLIA AND PSYCHASTHENIA

Case	Age	Hosp., days	Treatment, days	Maximum dose U	Sugar tolerance, hours			Result	Fasting blood sugar	Shock series	Total insulin U used
					1	2	3				
Melancholia											
1	62	58	41	80	336	203	110	Occup.	101	7	1880
2	69	112	96	70				Social	186	12	2110
3	75	25	18	40	101	280	194	Social	101	4	490
4	60	70	67	25	102	254	117	Social	104	10	980
5	55	92	45	25				Social	95	7	610
6	51	70	37	30				Occup.	81	5	635
Psychasthenia											
7	47	49	26	90	194	182	135	Occup.	118	4	1625
8	46	31	28	65				Occup.	101	4	740

and the dangers by ignoring the dietary factor. So the patients that indicated a high insulin tolerance by the character of their blood-sugar values were given 1820 calories each day in addition to the glucose necessary to terminate the shock. This diet was divided into 80 gm. of protein, 135 gm. of fat and 120 gm. of carbohydrate. The patients with average or low blood-sugar values were given a general diet containing 2100 calories of which 203 gm. was

carbohydrates until we could be certain that their insulin tolerance justified the use of the 1820 calorie diet.

Our first 6 patients received the regular insulin in divided doses, 2 or 3 times a day. Under this regime the insulin dosage was increased rapidly, shocks occurred at any time of the day or night and were difficult to control. Untoward reactions were numerous. Since then, we have found that much better control of the shocks was possible by giving the insulin in one dose at 5:00 A.M., starting with from 10 to 25 U and increasing the dosage from 5 to 10 U each day. As a rule the shocks were interrupted at 10:00 A.M. The subsequent insulin dose was always guided by the character of the shock and by the blood-sugar determination 5 hours after the insulin was given. Unless unfavorable reactions appeared, the insulin was given 6 days each week. In the event that severe reactions appeared, longer rest periods were used. These prolonged rest periods seemed to be indicated by serial shocks which occurred in 4 instances, by severe generalized convulsions which occurred in 10 per cent of our patients and by 7 instances of persistent tachycardia. Of these reactions, we had the greatest difficulty with the serial shocks that were similar to the after shocks reported by Reese and Vander Veer (3). They observed such reactions following the use of protamine zinc insulin. These shocks would recur about every second hour during the remainder of that insulin day in spite of repeated administration of glucose intravenously. These patients seemed incapable of absorbing glucose by the gastro-intestinal tract and intravenous glucose was of transitory value. One of these reactions occurred in *case 8* (table 3). An increase of her blood sugar to 243 mg. following the intravenous injection of 300 gm. of glucose over a period of 11 hours had a transient effect on her coma. These patients have remained in coma for as long as 18 days. To obviate these dangers we began to observe our daily blood-sugar readings critically. We found that the wet shock did not occur until the blood-sugar values were in the vicinity of 50 mg. We observed that when the 5-hour blood-sugar value reached that vicinity and the next daily reading dropped 20 mg. or more we could anticipate one of the severe reac-

TABLE 7. RESULTS

	Occupational	Social	None
	%	%	%
Entire Group	56	28	16
According to duration of illness			
Less than 6 months	69	24	7
Six to 12 months	25	25	50
More than 12 months	43	33	24
According to the mental disease			
Schizophrenia	60	19	21
Simple type	75		25
Hebephrenic type	50	33	17
Paranoid type	73	9	18
Catatonic type	33	33	34
Manic Depressive Psychosis	44	45	11
Manic phase	60	20	20
Depressed phase	25	75	
Melancholia	33	67	
Psychasthenia	100		

tions on the same insulin dosage within 48 hours. Since this 20 mg. drop of the blood-sugar presaged the severe reactions, we have used this sign as an indication to decrease the insulin dosage or increase the rest days. As a result of this change in management the last 26 patients of this series have rarely required intravenous glucose to terminate the shock.

The average number of series of shocks has been 7 or 42 shocks. The shortest series was 1 or 6 shocks and the longest was 17 or 102 shocks. Fifty per cent have had fewer than 42 shocks, 12 per cent required 12 shocks and 24 per cent had more than 66 shocks. The smallest total amount of insulin used was 80 U. On the average a total of 2072 U of insulin was necessary. To one patient we gave a total of 9255 U. The maximal dose given to the schizophrenia patients was 130 U in the simple type (table 1); 150 U in the hebephrenic (table 2); 90 U to the paranoid (table 3), and 130 U to the catatonic type (table 4). The maximal dose given to the manic depressive patients (table 5) was 80 U in the depressed phase and 75 U in the manic phase. The maximal dose used in melancholia patients was 80 U (table 6) and in psychasthenia patients was 90 U (table 6).

The kind of sugar used to terminate the shock did not seem to be a factor. We have found it necessary to use from 50 to 200 gm. of sugar by mouth or 10 to 300 gm. of glucose intravenously to terminate a shock. Glucose by rectum seemed valueless. In our first few patients the wet shocks were followed by fever, confusion and dry tongue. This apparently was due to chloride depletion since it has been controlled by giving 24 ounces of Ringer's solution daily by mouth throughout the period of insulin therapy. During the shocks we have found it possible to obviate the chance of bronchopneumonia by placing the patient on his side or face as soon as the shock began. Our experience has paralleled that of Reese and Vander Veer (4) in that we have not found adrenalin helpful in the severe reactions. Digitalis has been of little value in the control of tachycardia. We feel that it is of greater value to prevent these complications by the management of the therapy.

Concerning the systemic changes that have occurred, we have observed no detrimental effect upon the central nervous system excepting one instance of amentia and two instances of polyneuritis. Anorexia and difficult feeding problems were followed by normal or excessive appetites. During the severe reactions the gastro-intestinal mucosa seemed to lose its ability to absorb sugar. Hematemesis and melena were common at these times. Constipation was the rule. The pulse was accelerated persistently by the treatment in 7 of the patients. The other 47 patients had transitory tachycardia during their shocks, but as a rule they had a relative or actual bradycardia which became more apparent near and following the termination of the therapy. The treatments were usually followed by hypotension. Hadorn (5) has observed electrocardiographic evidence of transitory myocardial damage during insulin shocks. Our 7 instances of persistent tachycardia might have been due to such damage. We observed no physical evidence of such changes in the other patients, even in 2 of the patients who had pre-treatment evidence of chronic myocarditis.

Insulin therapy disturbed the endocrine mechanism but the changes in

function were usually slight and transitory. Some of these patients showed pancreas hypofunction. Glycosuria was common during the therapy. All of the patients had hyperglycemia on their rest days. Two patients had hyperglycemia with glycosuria for a month following the treatment. The early

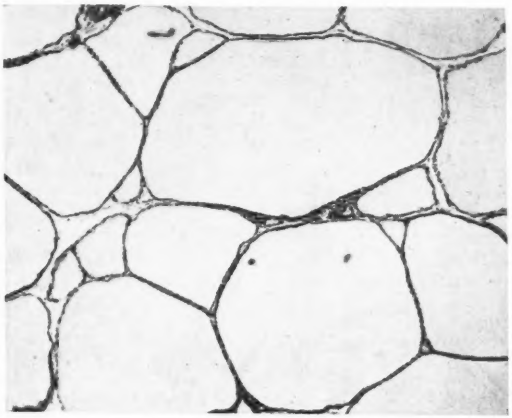


Fig. 1. Old nontoxic colloid goiter. There is no epithelial hyperplasia. The epithelium is of the flat type. The colloid spaces are very large. There is no increase in the interstitial connective tissue. *Case 1*, table 2.

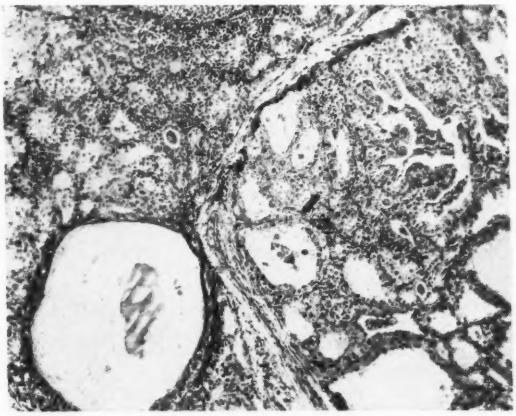


Fig. 2. Toxic goiter. There is marked proliferation of the epithelium with considerable papillary formation. The cells are of the columnar type. A number of the epithelial cells have desquamated into the colloid. Some of the acini seem disintegrated. There is a small amount of vacuolatd colloid material. *Case 8*, table 3.

cardiovascular and central nervous system excitability and the 7 instances of persistent tachycardia suggested adrenal hyperfunction. The profound post-treatment fatigue of several of the patients implied adrenal hypofunction. There were several instances of modified gonadal function. Sexual hyperactivity was usually diminished. Two of the females lost a pre-existing frigidity following the therapy. The menses were frequently decreased and 11 females

had amenorrhea during the treatment. We were unable to find signs of pituitary or parathyroid gland dysfunction.

The prolonged use of insulin in shock doses frequently decreased thyroid function. *Case 1* (table 2) had had a bilateral subtotal thyroidectomy on April 20, 1937. The gland had the appearance of an old colloid goiter as

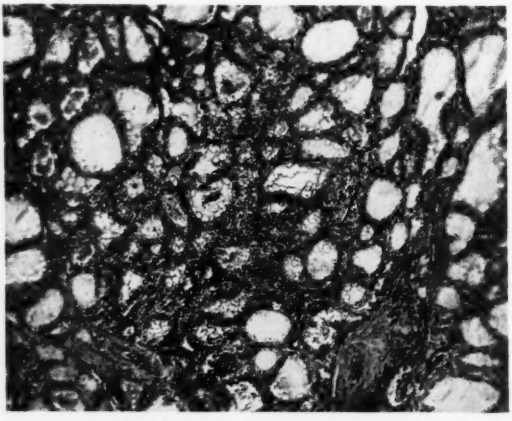


Fig. 3. Nodular toxic goiter. There is marked proliferation of the epithelium with some papillary development. The cells are of the columnar type and some of them have desquamated into the colloid. The colloid is decreased in amount and shows marked vacuolization. First lobe of *case 4*, table 3.

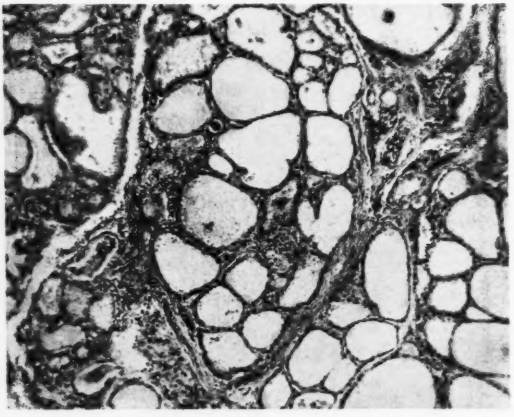


Fig. 4. Nodular toxic goiter. There is less epithelial proliferation and fewer papillary projections. The epithelium is flatter and the colloid spaces are larger. Second lobe of *case 4*, table 3.

shown in figure 1. She returned to the hospital on July 8, 1937 with a mild myxedema and schizophrenia. One grain of desiccated thyroid extract daily given until Aug. 28, 1937 controlled the myxedema but did not influence the psychosis. Insulin therapy was then given for her mental disturbance and although the thyroid extract therapy was continued she presented all the

symptoms and signs of a far advanced myxedema on the 24th day. Her basal metabolism rate reached —23 and was raised to +4 by the daily administration of 5 grains of thyroid extract. She still requires 1 grain of thyroid extract daily for maintenance. Six of the females and one male who had normal

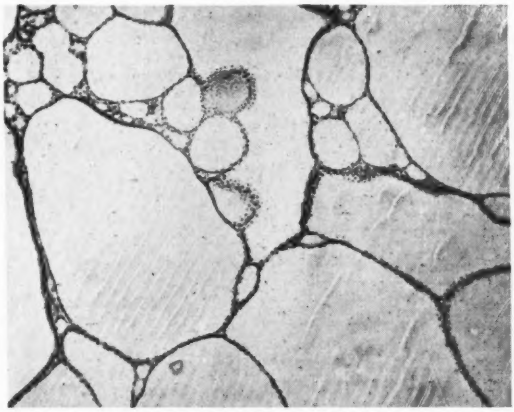


Fig. 5. Toxic goiter. There is little epithelial proliferation. Most of the cells are of the flat type. The colloid spaces are very large. There is no increase in the interstitial connective tissue. First lobe of *case 2*, table 6.

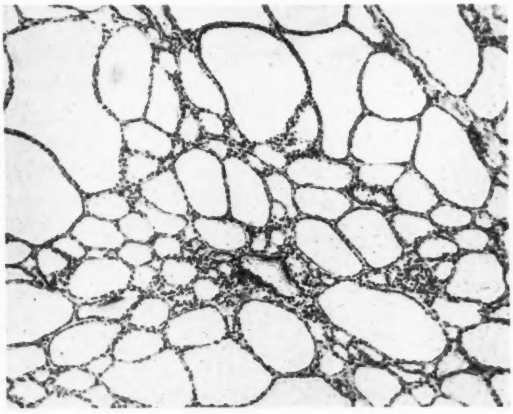


Fig. 6. Toxic Go'ter. There is a little more epithelial proliferation but the cells remain of the flat type. There are more interstitial cells and the colloid spaces are smaller. Second lobe of *case 2*, table 6.

thyroid glands and thyroid functions prior to insulin therapy developed mild post-treatment myxedema and required small maintenance doses of desiccated thyroid extract. *Case 8* (table 3) had treatment for a paranoid type of schizophrenia immediately following a subtotal thyroidectomy but she did not develop myxedema. Her gland was quite active as shown in figure 2.

Seven of the schizophrenia patients and 2 of the melancholia patients

had an associated thyrotoxicosis. Nervousness, loss of weight, tachycardia, excessive appetites, weakness, goiter, tremor and sweating were observed before the onset of their mental disturbances. Two of these patients had an associated myocarditis. A basal metabolic rate obtained on one of these, *case 4* (table 3) was +42. Their cardiac condition required that thyroidectomy be

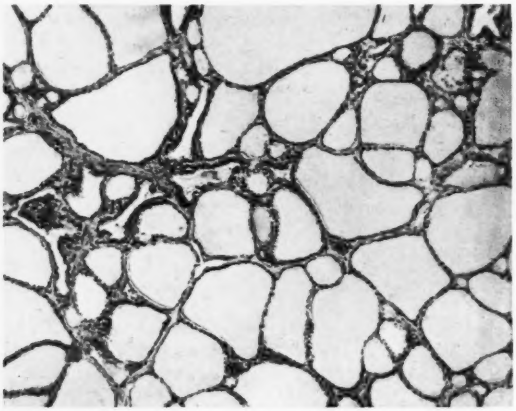


Fig. 7. Toxic Goiter. There is a slight amount of epithelial proliferation with some papillary formation. Most of the cells are of the flat type. There is a moderate amount of interstitial connective tissue. *Case 7*, table 1.

done by lobectomy stages. Both patients received insulin before each lobectomy. Their symptoms and signs of thyrotoxicosis diminished under insulin therapy. The goiter of one of them, *case 4*, (table 3) decreased in size. The size of the goiter of the other patient, *case 2*, (table 6) seemed unchanged. She had a complicating diabetes mellitus. The comparative microscopic inspection of the sections taken from the first lobe of *case 4* (table 3), as shown in figure 3, and of *case 2* (table 6), as shown in figure 5 and from the second lobe of *case 4* (table 3), as shown in figure 4 and of *case 2* (table 6), as shown in figure 6, showed decreased thyroid activity in the second specimen of the former and increased activity of the latter who had the complicating diabetes mellitus. *Case 7* (table 1) had had hyperthyroidism for 5 years. Under insulin therapy her basal metabolic rate dropped from +20 to +7 in 9 days. The symptoms and signs of the thyrotoxicosis and of the psychosis rapidly decreased and her reactions to the operation were normal. Microscopic section of her thyroid gland, figure 7, showed a moderately hyperactive glandular structure. Whether it was influenced by the brief period of insulin therapy is problematical. *Case 3* (table 3) had had a goiter for several years. On admission the gland was large and firm. Her pulse rate was 108 and her blood pressure 190/70. Her heart was enlarged. She had a fine tremor. During the previous 6 months she had lost 30 lb. in weight. Her basal metabolic rate was +30. After 27 days of insulin therapy her basal metabolic rate was +9. The goiter size and signs decreased. On dismissal her pulse rate was 72 and her blood pressure was 134/80. *Case 5* (table 2) had shown signs of hyperthyroidism

for a year. The goiter was large and firm. The basal metabolic rate was +32. Her weekly pulse rate average reached 103. After 139 days of insulin therapy the goiter was smaller, the signs of thyrotoxicosis markedly decreased and the weekly pulse rate average reached 73. Because of post-treatment mental dullness, a marked weight gain, dry skin and hair, bradycardia and hypotension, she has required 1 grain of desiccated thyroid daily for maintenance. The mental state of the 4 remaining patients of this group with goiters and signs of thyrotoxicosis did not permit the obtaining of basal metabolic rates during their preliminary study. Under insulin shock therapy their goiters decreased in size and the signs of thyrotoxicosis diminished or disappeared. Their weekly pulse rate averages decreased from 92 to 68 in *case 4* (table 1); from 98 to 68 in *case 7* (table 2); from 106 to 84 in *case 6* (table 6); and from 98 to 64 in *case 4* (table 2).

In conclusion, our results, as shown in table 7, were satisfactory. The variations were considerable, not only regarding the duration of the illness but also as concerning the mental diseases and their types. Our experiences with the early cases coincides with the reports of so many observers, that it needs no further comment. The outcome of the 6 to 12-month group was disappointing. Of those ill more than 12 months, the remissions were surprisingly frequent, and probably far exceeded the remission chances possible under non-shock forms of therapy. Six patients with the simple type of schizophrenia have made occupational recoveries. Two have not improved. Of the hebephrenic dementia praecox group, 7 have made occupational recoveries, 3 have reached a social recovery and 2 have not improved. The paranoid type has presented 6 occupational and 3 social grades of recovery. Two have not improved. Our catatonic group includes 2 occupational and 2 social recoveries and 2 failures. One of these failures made an occupational recovery about 6 months after treatment was discontinued. The manic depressive group results included 3 occupational and 1 social recovery and 1 failure of those treated during the manic phase. Four patients treated in the depressed phase resulted in 1 occupational and 3 social recoveries. Two occupational and 4 social recoveries were the results of the treatment of melancholia. Both of the patients with psychasthenia have made occupational recoveries, but their morbid tendencies seem to have been submerged rather than erased. Seven of the patients with an associated thyrotoxicosis have made occupational recoveries and the other 2 have made social recoveries. Three patients have had relapses, 2 of them have had more satisfactory grades of remissions following their second series of insulin shock therapy than they obtained after their first course. Both of these were of the hebephrenic type of schizophrenia. The third had melancholia and is now undergoing her second series of treatment.

SUMMARY

We found Sakel's insulin shock therapy of definite value in the treatment of schizophrenia. In the following order it was helpful in the paranoid, simple, hebephrenic and catatonic types. It was of equal value in the treatment of patients with the manic phase of manic depressive psychosis. It was helpful

in psychasthenia. Its value was questionable in the depressed phase of manic depressive psychosis and in melancholia. The character of the preliminary sugar curves or the fasting blood-sugar levels was of no value in predicting the results. The blood sugar determinations were of definite value in the pretreatment consideration and of great value in the management of the therapy. After the capillary blood-sugar reading reached the shock level of the 50 mg. vicinity, a comparative 24-hour drop of 20 mg. or more was a danger sign of considerable value. We feel that part of the danger encountered in insulin shock therapy can be controlled by means of a low carbohydrate diet, thus avoiding the need of larger doses of insulin. We could observe no immediate or late changes in the parathyroid or pituitary gland functions. Pancreas hypofunction was transitory. The therapy seemed to cause early and in some patients prolonged adrenal hyperfunction or hypofunction of a minor character. There was a tendency to modified gonadal function. It frequently caused early and persistent decrease of the thyroid gland function, sufficient to require thyroid extract therapy in 8 patients. Thyrotoxicosis was decreased in 9 patients. We did not observe evidence of permanent visceral damage. The therapy caused major central and peripheral nervous system changes in 3 patients. In one of these the damage may be permanent. We had no deaths.

REFERENCES

1. Sakel, Manfred: Neue Behandlung der Schizophrenie, Vienna, M. Perles, 1935.
2. Cowie, D., J. P. Parsons and Theo. Raphael: J. Michigan M. Soc. **22**:383. 1923.
3. Reese, Hans H., and Adrian Vander Veer: Arch. Neurol. & Psychiat. **39**:232. 1938.
4. Reese, Hans H., and Adrian Vander Veer: Arch. Neurol. & Psychiat. **39**:702. 1938.
5. Hadorn, W.: Arch. f. Kreislaufforsch. **2**:70. 1937.

THE EFFECT OF TESTOSTERONE PROPIONATE ON THE GENITAL TRACT OF THE IMMATURE FEMALE RAT

UDALL J. SALMON
From the Laboratories of the Mount Sinai Hospital
NEW YORK CITY

Butenandt and Kudzus (1) have noted that certain androgens, when injected into immature female rats, cause premature opening of the vagina. This observation has been interpreted as indicating that these androgens possess, by virtue of similarity in chemical structure to the estrogens, estrogenic properties (2). There is, furthermore, some experimental evidence to indicate that certain of the androgens may be converted in the body to an estrogenic substance (3). In contrast to these experimental investigations we find that the estrus cycle can be suppressed by means of testicular extracts (4), and by testosterone (5, 6). Korenchevsky and his co-workers (7), Nelson and Gallagher (8) and others, have shown that androgens cause hypertrophy of the uterus and vagina of adult spayed, as well as intact, rats. Nelson and Merckel (3) have observed that testosterone, androsterone, dehydroandrosterone and androstanedione failed to produce vaginal cornification in spayed rats. However, testosterone and androstanedione produced enlargement of the uterus, whereas androsterone and dehydroandrosterone had no effect on the uterus. Hartman (9) has reported that menstruation is suppressed in monkeys after testosterone injections. Zuckerman (10) has shown that in monkeys testosterone propionate inhibits follicle maturation and luteinization, resulting in suppression of the menses. Cotte, Martin and Mankiewicz (11) reported similar results in rabbits. In a recent report (12) it was shown that testosterone propionate and androstenediol cause follicle growth and corpora lutea formation in the ovaries of immature rats. This observation was interpreted as indicating that the premature opening of the vagina which occurred in infantile rats as a result of androgen administration was probably the result of a primary stimulation of the hypophysis resulting in a gonadotropic effect upon the ovary with consequent estrogen and progesterone production by the ovary. The present investigation was undertaken to determine the effect of testosterone propionate upon the various components of the female genital tract of the immature rat.

EXPERIMENTAL PROCEDURE

Litter-mate rats (27 to 30 days old) were given doses of testosterone propionate varying from 0.5 to 20 mg., over periods varying from 1 to 30 days. In one series of experiments a number of animals of the same litter were spayed before being injected. The time of opening of the vagina was noted

in each case and vaginal smears were taken daily until 4 weeks after the control animals had developed spontaneous estrus. At intervals varying from 24 to 240 hours, after the injections were begun, the animals were sacrificed and sections made of the ovaries, uterus, vagina and preputial glands.

RESULTS

Effect of testosterone propionate on the vagina. In the animals receiving single injections of testosterone propionate, vaginal opening occurred with doses varying from 0.5 to 20 mg., and as early as 72 hours after injection. Vaginal smears taken at the time when the vagina was first noted to be open revealed, in most instances, a full estrus effect. This disappeared within a day. Histologic examination of the vagina at this time revealed marked epithelial proliferation and in some areas slight cornification (fig. 1 and 2). The smears thereafter showed either mucus only, or occasionally a few scattered epithelial cells. This type of smear continued until the time when the uninjected control animals developed estrus reactions. The smears taken on the injected animals thereafter revealed estrus cycles similar to the controls.

The animals receiving continuous injections showed cornified smears for periods varying from 5 to 8 days. Thereafter, in spite of the continued administration of testosterone propionate, the smears consisted of mucus with a few small epithelial cells. Following the discontinuation of the injections the vaginal smear during the following 20 to 32 days contained only mucus. After this period of mucus smears, a normal estrus vaginal cycle occurred.

The vaginas of the spayed immature rats opened somewhat later than in the intact litter mates receiving the same dosage of androgen. The difference in time varied in different litters from 6 to 16 hours.

Effect of testosterone propionate injections on the ovaries. It has been previously reported (12) that evidence of follicle growth was noted as early as 60 hours after the administration of testosterone proprionate or androstenediol, and corpora lutea as early as 96 hours. Sections of ovaries removed from the animals after longer periods of injections (10 to 20 days), with total doses varying from 8 to 20 mg., all revealed corpora lutea (fig. 3 to 5).

Effect of testosterone propionate injections on the uterus. Uteri removed as early as 72 hours after a single injection of 1 to 5 mg. of testosterone propionate showed marked enlargement with hypertrophy of the musculature and marked proliferation of the mucosa (fig. 7). Castrated immature female litter mates injected with the same amount responded with less marked enlargement of the uterus (fig. 8).

Effect of testosterone propionate on preputial glands. Korenchevsky and his co-workers (15) have drawn attention to the enlargement of the preputial glands following androgen injections in adult female rats. In this series, enlargement of the preputial glands was noted as early as 48 hours after the first injection. The weight of the control preputial glands varied from 10 to 16 mg. The weight of the glands in the immature rats following testosterone propionate administration varied from 35 to 88 mg. (fig. 10 and 11). Enlargement of the terminal end of the urethra, caused by down-growth of the pre-

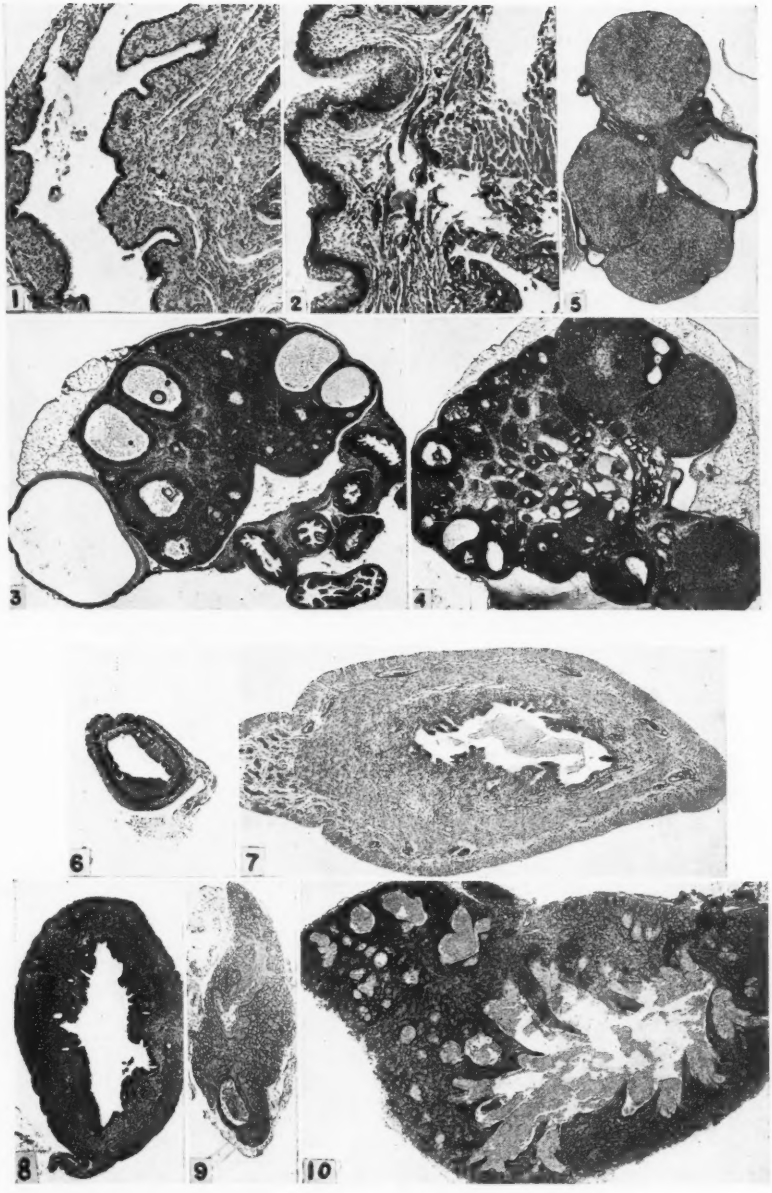


Fig. 1. Vaginal mucosa of control immature rat aged 30 days.
Fig. 2. Vaginal mucosa of litter mate 72 hours after 2 mg. of testosterone propionate.
Fig. 3. Follicle stimulation in litter mate, 72 hours following 2 mg. testosterone propionate.
Fig. 4. Corpus-luteum formation in litter mate 120 hours after 2 mg. testosterone propionate.
Fig. 5. Ovary of litter mate 8 days after 10 mg. testosterone propionate.
Fig. 6. Uterus of control immature rat aged 30 days.
Fig. 7. Uterus of litter mate 72 hours after 5 mg. testosterone propionate.
Fig. 8. Uterus of spayed litter mate 72 hours after 5 mg. testosterone propionate.
Fig. 9. Preputial gland of control immature rat aged 30 days.
Fig. 10. Preputial gland of litter mate 72 hours after 5 mg. testosterone propionate.

putial glands, is noticeable even before opening of the vagina occurs. By exerting a little pressure on each side of the urethra, a thick subaceous secretion exudes from the meatus. Presence of the secretion has been noted as early as 36 hours after a single injection of 5 mg. of testosterone propionate. A minute amount of secretion can occasionally be expressed from the urethra

Fig. 11. Internal genitalia of rat aged 34 days, 96 hours after 5 mg. testosterone propionate, demonstrating enlargement of ovaries, hypertrophy of uterus and preputial glands. P = preputial gland.

of the controls, but this secretion is thin in consistency and whitish in color. Similar enlargement of the preputial glands was noted in ovariectomized immature rats.

SUMMARY

Testosterone propionate administered to immature female rats causes premature opening of the vagina followed by a period of estrus reaction lasting 1 to 2 days. If no more injections are given, the vaginal smears contain only mucus or occasionally a few small epithelial cells up to the time of normal spontaneous onset of estrus. The estrus cycle, once it appears, is normal. If testosterone propionate injections are continued, the vaginal smears show an estrus reaction for 5 to 8 days. Thereafter, in spite of continued injections, the smears show only mucus and a few small epithelial cells.

Testosterone propionate causes follicle growth and corpora-lutea formation in the ovaries of immature female rats. It causes marked enlargement of the uterus of immature rats, the enlargement consisting of hypertrophy of the musculature and proliferation of the endometrium. It causes marked enlargement of the preputial glands of immature female rats with retention of an abundant secretion which can be expressed even before opening of the vagina occurs.

In ovariectomized immature rats, testosterone propionate causes enlargement of the uterus and preputial glands and opening of the vagina. The uterine enlargement is, however, less marked than that in the intact immature rats.

It is suggested that the mechanism of testosterone propionate action in immature female rats consists of a combination of two factors, namely, a direct effect upon the uterus and vagina as well as an indirect one through the hypophysis and ovaries. The more marked enlargement of the uterus which occurs in intact animals after testosterone propionate injections, as compared with castrates, is probably due to the combined action of the two components—the direct estrogen effect of testosterone propionate and an estrogen-progesterone effect—the latter being supplied by the rat's own ovaries.

It appears from these results that testosterone propionate when administered to immature female rats has a stimulating trophic effect not only upon the components of the female genital tract but also upon the male vestiges, namely, the preputial glands, which are considered to be the homologues of the prostate.

REFERENCES

1. Butenandt, A., and H. Kudzus, Hoppe-Seyler's Ztschr. f. physiol. Chem. **75**:237. 1935.
2. Deanesley, R., and A. S. Parkes: Brit. Med. J. **1**:257. 1936.
3. Nelson, W. O., and C. G. Merckel: Proc. Soc. Exper. Biol. & Med. **36**:823. 1937.
4. Ihrke, I. A., and F. E. D'Amour: Am. J. Physiol. **96**:289. 1931.
5. Robson, J. M.: Proc. Soc. Exper. Biol. & Med. **35**:49. 1936.
6. Browman, L. G.: Proc. Soc. Exper. Biol. & Med. **36**:205. 1937.
7. Korenchevsky, V., M. Dennison and K. Hall: Biochem. J. **31**:780. 1937.
8. Nelson, W. O., and T. F. Gallagher: Science **84**:230. 1936.
9. Hartman, C. G.: Proc. Soc. Exper. Biol. & Med. **37**:87. 1937.
10. Zuckerman, S.: Lancet **2**:676. 1937.
11. Cotte, G., J. F. Martin and E. Mankiewicz: Gynécologie **36**:561. 1937.
12. Salmon, U. J.: Proc. Soc. Exper. Biol. & Med. **37**:488. 1937.

THE USE OF TESTOSTERONE PROPIONATE IN FUNCTIONAL BLEEDING

SAMUEL H. GEIST, UDALL J. SALMON AND JOSEPH A. GAINES
From the Gynecological Service and the Laboratories of the Mount Sinai Hospital
NEW YORK CITY

The present communication is a report on the use of testosterone propionate (male sex hormone) in functional uterine bleeding. Of 25 cases so treated, the excessive bleeding was rapidly controlled in all but two. This clinical improvement was correlated with definite changes in the endometrium, as revealed by suction biopsies performed at intervals before, during and after treatment.

The rationale for this form of therapy has its basis in animal experimentation as well as in our studies on the effect of testosterone propionate on the endometrial cycle of the human. Suppression of the estrus cycle in female rats was first induced with male hormone extracts of bull testes by Ihrke and D'Amour (1). Similar results were obtained with testosterone by Robson (2) in mice and Browman (3) in rats. In monkeys, Hartman (4) has shown that menstruation was delayed following testosterone administration, and Zuckerman (5) has reported that testosterone propionate inhibits follicle stimulation and luteinization, resulting in suppression of the menses. Cotte, Martin and Mankiewicz (6) reported similar results in rabbits. In a previous report (7) we have shown that administration of adequate doses of testosterone propionate causes striking morphologic changes in the endometrium of regularly menstruating women, associated with suppression of the succeeding menstrual period. These changes consist of the disappearance of the secretory phase and inhibition of the proliferative phenomena. The inhibition of proliferative activity in the endometrium, together with the suppression of the menses, suggested the use of male hormone in functional uterine bleeding. The hormone selected for this investigation was synthetic testosterone propionate inasmuch as it has been shown to be the most potent preparation available (8, 9).

Twenty-five cases of abnormal uterine bleeding were chosen for this study. In all but 4 there was no palpable organic disease; in the 4, small intramural myomata were present. Accurate graphic records were kept of the amount and duration of bleeding. In all but 2 cases one or more pre-treatment suction biopsies of the uterine endometrium were taken for comparison with curettings removed during and after treatment. In some instances, as many as 9 suction curettages were performed during the period of observation, in order to follow closely the endometrial changes resulting from the testosterone administration. The curettings were stained with hematoxylin and eosin and

also with a modified Best Carmine stain for glycogen. The periods of observation, calculated from the beginning of treatment, varied from 2 to 10 months.

There were 13 cases of menorrhagia, 5 of meno-metrorrhagia and 7 of menorrhagia with poly-menorrhea. Fifty per cent of the patients were between 40 and 49 years of age; the others varied from 24 to 39 years of age. The

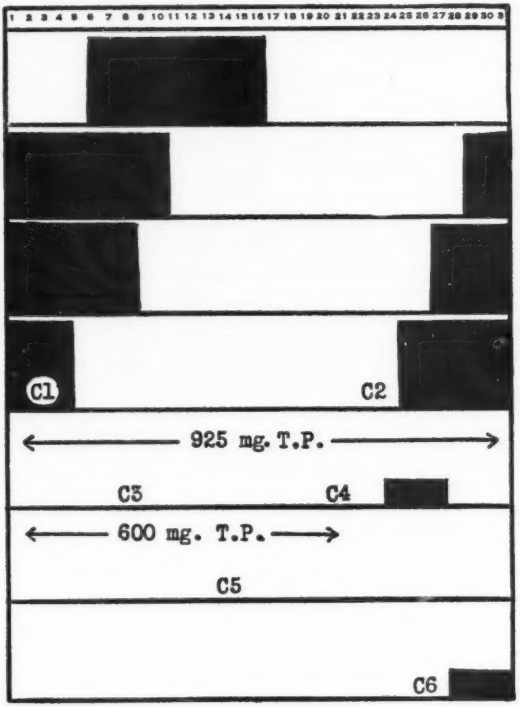


Fig. 1. *Case 1.* H. S., aged 34 years. Menorrhagia for 10 years. Short period of amenorrhea and normal menses after the use of testosterone propionate. Note return of secretory endometrium after the cessation of treatment. C1 = secretory (menstrual phase—). C2 = advanced secretory phase. C3, C4 and C5 = atrophy. C6 = secretory phase. C = endometrial biopsy. T.P. = testosterone propionate.

duration of abnormal bleeding varied from 2 months to 10 years with an average of 2 years.

Dosage. In the early part of the investigation, because of uncertainty as to the optimal dosage, patients were started with amounts varying from 45 to 200 mg. per month. It soon became apparent that in most cases larger doses were necessary to achieve a therapeutic effect. The observation had previously been made that as much as 815 mg. could be given to a female castrate during one month without untoward results (10). In view of this, the total monthly dosage was increased in some instances to 1000 mg. The highest dosage given any single case was 2150 mg. over a period of 3 months. The

testosterone propionate[1] was administered intramuscularly in sesame oil in doses of 5 to 100 mg.

Preliminary endometrial biopsies. Control curettings were secured premenstrually in most instances, in an attempt to determine the presence of a corpus-luteum effect. At intervals, additional biopsies were obtained during the period of bleeding and during other phases of the cycle. In 19 of the 25 cases, the abnormal bleeding occurred from an endometrium in the secretory

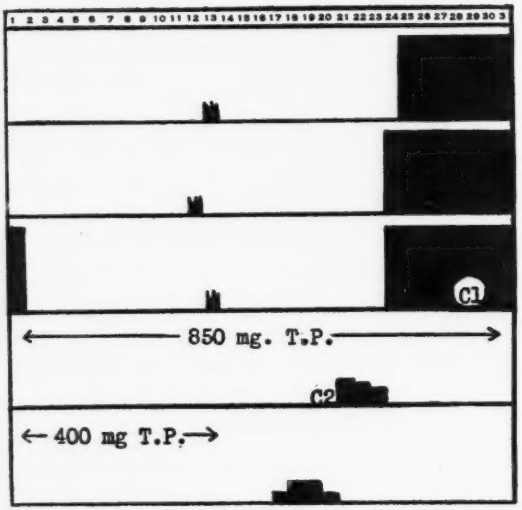


Fig. 2. *S. W.*, aged 37 years. Menorrhagia for 6 months. Establishment of normal menses without the inhibition of the secretory phase. Note abolition of spotting at time of ovulation. C1 menstrual phase with secretory epithelium. C2 advanced secretory phase.

phase. In 3, a proliferative endometrium was obtained on the 7th, 16th and 21st day of the cycle, respectively. In 2 instances, a typically hyperplastic endometrium was found.

Diagnostic criteria. Because of the lack of uniformity in the terminology employed in the literature to describe the morphologic variations of the endometrium, a brief description is presented of the histologic criteria and classification followed in this study. The normal monthly cycle is divided into menstrual, proliferative and secretory phases. The proliferative phase occurs from the end of menstruation to the time of ovulation. It is generally accepted to be the result of a purely estrogen stimulation of the endometrium, manifested in progressive cellular activity and growth. The secretory phase occurs from the time of ovulation to the beginning of menstruation and is the result of a combined estrogen and progesterone effect. A distinctive characteristic of this phase, of value in differentiating it from the proliferative phase, is the presence of large amounts of glycogen within the epithelial cells. The term hyperplastic endometrium indicates an abnormally marked estrogen effect and ex-

[1] For the testosterone propionate used in this investigation, we are indebted to Dr. Erwin Schwenk, Schering Corporation, and Mr. Robert C. Mautner, Ciba Pharmaceutical Products.

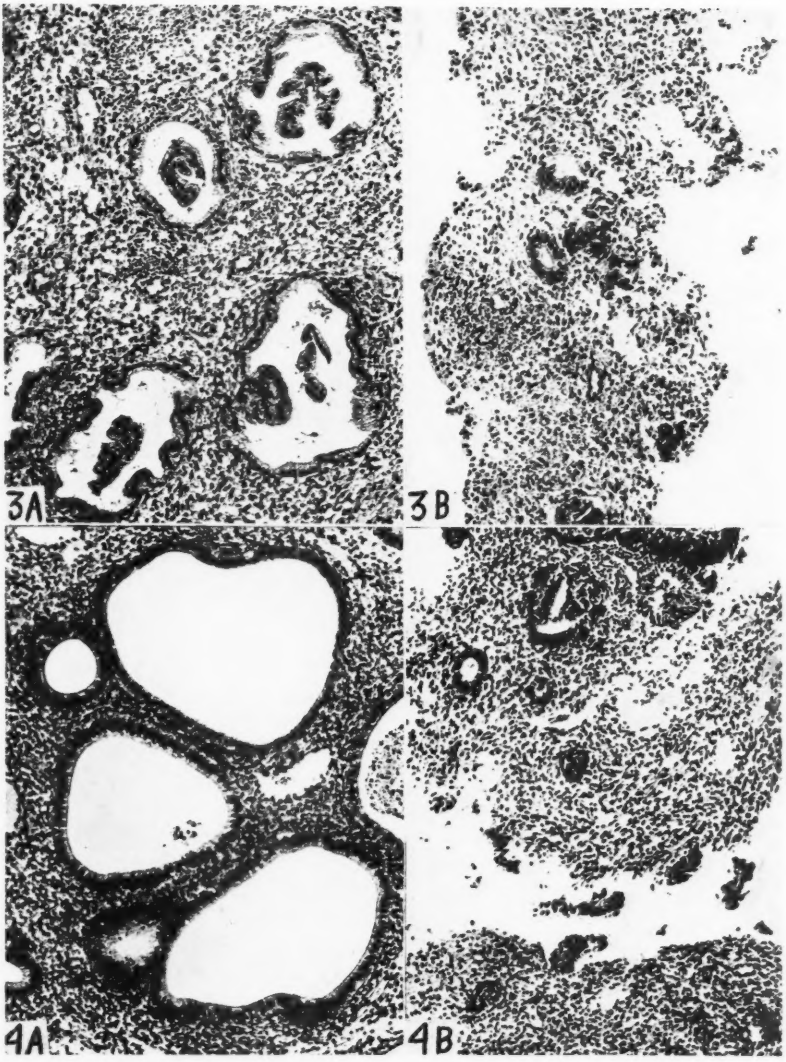


Fig. 3*A*. *Case 1*. C-2. Preliminary suction curettage taken one day pre-menstrually. Typical secretory phase.

Fig. 3*B*. *Case 1*. C-4. Suction biopsy of endometrium one month later, after administration of 675 mg. testosterone propionate. Atrophic endometrium.

Fig. 4*A*. *O. M.*, aged 49 years. Meno-metrorrhagia for 2 years. Hyperplastic endometrium obtained during a 2-week period of bleeding.

Fig. 4*B*. Cessation of bleeding after 90 mg. testosterone propionate, followed by amenorrhea of 5 months. Under continued therapy (750 mg. testosterone propionate during 3½ months of amenorrhea), reduction of endometrium to state of atrophy.

cludes the presence of any corpus-luteum reaction. It represents a more marked degree of proliferation than that seen in the late proliferative phase of the normal menstrual cycle.

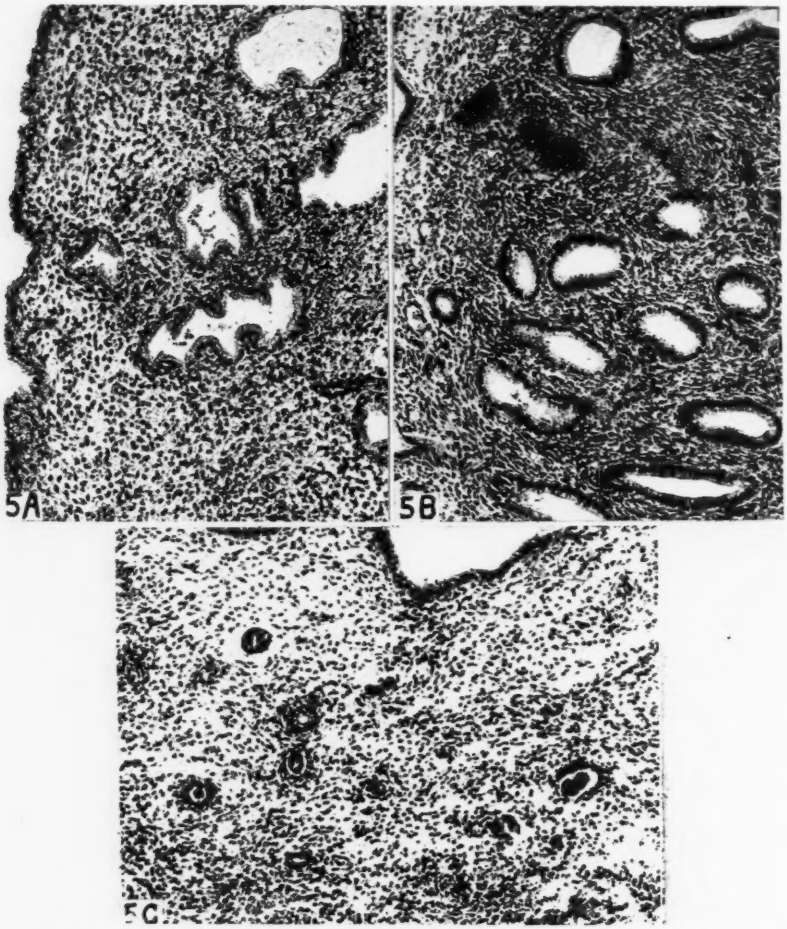


Fig. 5*A*. *C. V.*, aged 40 years. Menorrhagia for 4 months. Preliminary curettage one day before onset of menses showing secretory phase.
Fig. 5*B*. One month later, after 800 mg. testosterone propionate. No bleeding. Endometrium showing moderate proliferation.
Fig. 5*C*. Two months later; additional 300 mg. testosterone propionate given. Endometrium showing hypoplasia.

The endometrial changes noted after the administration of testosterone propionate in normal cyclical women appear to be the reverse of those observed in hyperplasia. In contrast, they suggest various degrees of inhibition or suppression of endometrial proliferation. The following terms have been proposed to designate gradations of subnormal proliferation; in other words, less than that seen in the early proliferative phase of the normal cycle: *a*) slight or marked hypoplasia, when the cellular activity and growth are strikingly reduced; and *b*), atrophy, when the endometrial picture resembles that found long after the menopause or castration. A description of the histologic

details of endometrial hypoplasia and atrophy have been reported elsewhere (7).

CLINICAL RESULTS

Definite clinical improvement occurred in all but 2 cases. In 14 of the 25 cases, a temporary amenorrhea of 2 to 5 months' duration occurred. Of these, 9 subsequently had normal menses. Up to the present writing, 3 of the remaining 5 missed one period, and the others 2 and 5 periods respectively. In 9 cases, normal menses were established without interrupting their regularity. In 2, the menses were delayed but normal in amount, and in 2 others they were regular but very scanty.

Clinical Results in 25 cases of Meno-metrorrhagia Treated with Testosterone Propionate

Normal Menses	Amenorrhea (1-3 months) followed by normal menses	Amenorrhea (1-5 months to date)	No Effect
9 cases	9 cases	5 cases	2 cases

A sufficient number of cases have not been followed for a long enough period after treatment to warrant final conclusions as to the duration of the therapeutic effect. However, it appears from the data available that normal ovulatory menstruation ultimately occurs after treatment in those cases in which the preliminary examination had revealed a progesterone effect pre-menstrually. This appearance of the endometrium showing a normal estrogen-progesterone effect was noted even after a period of induced amenorrhea of 5 months' duration. The following case illustrates this point.

Case 1. H. S., (fig. 1) a 34-year-old negro woman, complained of severe menorrhagia. Normal menstrual periods had occurred until 10 years ago. Since then the menses, although regular, had become prolonged 7 to 15 days, with profuse flow and passage of clots. Nine years ago x-ray therapy was administered at another institution without change in the menorrhagia. Five years ago a dilation and curettage was performed on 2 occasions without relief. Pelvic examination revealed no abnormalities of vagina, cervix, uterus or adnexa. Preliminary suction biopsies obtained during a bleeding period and pre-menstrually showed a menstrual and secretory phase, respectively (fig. 3*A*). Treatment with testosterone propionate was started immediately after the menses. On the 13th day of the cycle, after 1 week of concentrated therapy (300 mg.), an atrophic endometrium was obtained. At the end of 27 days, during which a total of 675 mg. (fig. 3*B*) had been administered, the endometrial picture remained that of atrophy. On the 30th day, 3 days after the previous curettage, uterine bleeding of normal amount occurred for a period of 4 days. During the next month, 850 mg. of testosterone propionate was given. Atrophy of the endometrium continued, accompanied by an amenorrhea of 2 months. Five weeks after the discontinuation of treatment, suction curettage showed a secretory phase. Two days later, a normal menstrual period of 4 days' duration occurred.

The history which follows illustrates the effect of testosterone propionate in a case of functional bleeding associated with hyperplasia of the endometrium. In this instance, normal bleeding from a proliferative endometrium occurred after the cessation of treatment.

Case 2. S. R., aged 46 years, complained of meno-metrorrhagia of 3 years' duration. A curettage, performed one year ago, revealed hyperplastic endometrium. The meno-metrorrhagia continued unaffected by the curettage. When seen in our clinic,

Aug. 22, 1937, she reported that she had been bleeding profusely and steadily for 5 weeks. A suction curettage performed, while she was still bleeding, showed hyperplastic endometrium. The patient was given 2 injections of 25 mg. each of testosterone propionate and the bleeding ceased. The testosterone propionate injections were continued in amounts of 5-15 mg. twice weekly for 5 months. During the 5 months of treatment and for 2 months thereafter, the patient did not menstruate. This period of amenorrhea was followed by 3 menstrual periods which were slightly subnormal in the amount of flow, at intervals of 26, 24 and 29 days. During the course of treatment and amenorrhea, 5 biopsies showed regression of the proliferative activity, indicated particularly by the loss of pseudostratification of the epithelium and marked diminution of the number of mitotic figures. After the patient had begun to menstruate normally, 2 endometrial biopsies presented a picture of moderate proliferation without evidence of corpus-luteum effect. In another case of endometrial hyperplasia (fig. 4*A* and 4*B*), the endometrium was reduced to a state of atrophy with larger doses (840 mg.).

The two cases that were considered failures merit detailed description.

Case 3. A. B., aged 41 years, gave a history of menorrhagia of 1 year's duration. A control permenstrual curettage revealed a secretory endometrium. The patient was given 450 mg. of testosterone propionate from the 7th day to the 18th day of the cycle. The next menstrual period occurred at the expected time and was slightly more profuse than the one preceding treatment. Unfortunately, the patient refused further treatment.

Case 4. B. S., aged 43 years, complained of meno-metrorrhagia of 3 years' duration. A therapeutic curettage had been performed 3 months previously without amelioration of the bleeding. Pretreatment curettage, performed on the day before menstruation, revealed a late secretory phase. Testosterone propionate injections were begun on the first day of the period and continued 3 times weekly for 6 weeks, a total of 1375 mg. being given. Clinically, an amenorrhea of 2 months duration resulted with regression of the endometrium to slight proliferation. Four weeks after discontinuation of treatment, profuse bleeding began which lasted 3 days. This patient is being kept under observation to determine the character of the subsequent menses.

The problem of determining the minimal therapeutic dose that will control the bleeding is a difficult one. There is apparently an individual variation in the response to the hormone. However, the dose required to produce a therapeutic effect seems to vary approximately from 300 to 800 mg. per month. In several cases we have observed lack of response to smaller doses, whereas increasing the dosage produced a striking effect both on the endometrium and on the bleeding. This is illustrated by the following case.

Case 5. H. L., aged 24 years, gave a history of polymenorrhea and menometrorrhagia of 16 months' duration. Premenstrual endometrial biopsy revealed the endometrium to be in the secretory phase. 100 mg. in the first month and 145 mg. in the 2nd month were insufficient to influence either the bleeding or the endometrium. When the dose was increased to 275 mg., the secretory phase was suppressed, but bleeding was not significantly affected. Increasing the dosage to 600 mg, in the following 4 weeks resulted in suppression of the menses for 2 months and regression of the endometrium to a state of hypoplasia. A curettage

3 weeks after the cessation of treatment showed evidence of active proliferation, indicating initial regeneration.

Some side effects of testosterone propionate are worthy of note. Ten of the cases volunteered the information that they felt more vigorous and had increased appetite. In 8 of these, a definite gain in weight was apparent. Four cases developed mild facial hirsutes. These were brunettes with a slight, but definite, hirsutes before treatment. One case developed a facial acne. In 3 cases, slight coarsening of the voice was noted. After the discontinuation of treatment, these clinical by-effects showed signs of regression, with the exception of one case of hirsutes.

COMMENT

The question of the modus operandi of testosterone propionate is an intriguing one. Animal experimentation has shown that whereas androgens inhibit estrus (2, 3, 11), and ovulation (5, 6) there is also evidence that they have a stimulating trophic effect on the genital tract. Thus Korenchevsky (12), and his co-workers, and Nelson and Gallagher (17), have shown that androgens cause hypertrophy of the uterus in adult intact as well as spayed rats. Butenandt and Kudzus (13) have shown that some androgens cause premature opening of the vagina in immature rats, and Salmon (14) has recently reported that testosterone propionate and androstenediol produce a gonadotropic effect (follicle stimulation and corpora-lutea formation) in immature rats. To add to the complexity of the problem, Klein and Parkes (15) have also shown that testosterone has a progesterone-like action on the uterus of the castrated rabbit. It is difficult to interpret our observations in humans in the light of these complex and, in some respects, paradoxical reactions in animals.

Reports of the use of male hormone in human females are few in number. It has been shown in a human castrate (10) that the hypophysis can be inhibited and the castration symptoms concomitantly relieved with testosterone propionate. Loeser (16) has reported improvement in the bleeding in 14 cases of menorrhagia following treatment with testosterone propionate. In a previous report (7), describing the inhibitory action of testosterone proprionate upon the endometrium of human cyclical females, we made the suggestion that testosterone may prevent the development of normal endometrial proliferative and progestational changes through inhibition of the gonadotropic factors of the hypophysis, resulting in suppression of the normal ovarian cycle. This would explain the amenorrhea and the associated suppression of estrogen and progesterone effects on the endometrium.

By the same token it seems logical to assume that in the cases of functional bleeding (regardless of the type of pre-treatment endometrium found), the mechanism of the control of the menorrhagia is a similar one, namely, primary inhibition of the gonadotropic factors of the hypophysis with secondary suppression, in varying degrees, of estrogen and progesterone formation.

SUMMARY

The effect of testosterone propionate was studied in 25 cases of abnormal uterine bleeding. In 21 of these, no palpable evidence of organic pelvic disease was found. In 4, examination revealed small intramural myomata. Suc-

tion curettages were performed before treatment and revealed, in 18, a secretory phase pre-menstrually; in 2, hyperplasia of the endometrium; and in 3 others, a proliferative phase during the 7th, 16th and 21st day of the cycle, respectively. With doses varying from 300 to 1000 mg. per month, the excessive bleeding was controlled in all but 2 cases. In 18 cases normal menses were established; in 5, amenorrhea of 1 to 5 months' duration up to the present time. In 2 cases, the amount of bleeding was not affected. Endometrial biopsies performed during and after the period of testosterone propionate administration revealed the following morphologic changes: *a*), Disappearance of the secretory phase (85%); *b*), Inhibition of the proliferative phase, often with regression to the hypoplastic or atrophic state. Within a month following the cessation of treatment, the endometrial biopsies revealed evidence indicating a beginning regeneration during the period of amenorrhea.

Testosterone propionate (in doses of 300-1000 mg. per month) inhibits menstruation and arrests the endometrium at the early proliferative phase, preventing the development of progestational changes. Larger doses cause varying degrees of regression of the endometrium to the state of hypoplasia or atrophy. Following the discontinuation of therapy, the inhibitory effects on the endometrium gradually disappear and normal estrogen and progesterone effects reappear. It is suggested that the regressive changes noted in the endometrium after testosterone propionate administration are the end results of a primary inhibition of the gonadotropic factors of the hypophysis, causing suppression of the ovarian cycle, with consequent cessation of estrogen and progesterone production.

REFERENCES

1. Ihrke, I. A., and F. E. D'Armour: Am. J. Physiol. **96**:289. 1931.
2. Robson, J. M.: Proc. Soc. Exper. Biol. & Med. **35**:49. 1936.
3. Browman, L. G.: Proc. Soc. Exper. Biol. & Med. **36**:205. 1937.
4. Hartman, C. G.: Proc. Soc. Exper. Biol. & Med. **37**:87. 1937.
5. Zuckerman, S.: Lancet **2**:676. 1937.
6. Cotte, G., J. F. Martin and E. Mankiewicz: Gynécologie **36**:561. 1937.
7. Gaines, J. A., U. J. Salmon and S. H. Geist: Proc. Soc. Exper. Biol. & Med. **38**:779. 1938.
8. Miescher, K., A. Wettstein, E. Tschopp: Biochem. J. **30**:1970. 1936.
9. Parkes, A. S.: Lancet **2**:674. 1936.
10. Salmon, U. J.: Proc. Soc. Exper. Biol. & Med. **37**:488. 1937.
11. Nelson, W. O., and C. G. Merckel: Proc. Soc. Exper. Biol. & Med. **36**:823. 1937.
12. Korenchevsky, V., and K. J. Hall: Path. and Bact. **45**:681. 1937.
13. Butenandt, A., and H. Kudzus: Hoppe-Seyler's Ztschr. f. Physiol. Chem. **75**:237. 1935.
14. Salmon, U. J.: Proc. Soc. Exper. Biol. & Med. **38**:352. 1938.
15. Klein, N., and A. S. Parkes: Proc. Roy. Soc. **121**:574. 1937.
16. Loeser, A. A.: Lancet **1**:373. 1938.
17. Nelson, W. O., and T. F. Gallagher: Science **84**:230. 1936.

THE INFLUENCE OF ESTROGENS IN EGG YOLK UPON AVIAN BLOOD CALCIUM

M. ALTMANN AND F. B. HUTT
From the Department of Poultry Husbandry, Cornell University
ITHACA, NEW YORK

Following the demonstration by Riddle and Reinhart (1) that in pigeons and doves there is a marked increase in blood calcium as the females come into laying, it has been shown by a number of workers that a similar situation prevails in the fowl. Whereas the level of blood calcium in non-laying females and males of that species is usually less than 15 mg. per 100 cc. serum, in laying fowls blood calcium is maintained at from 15 to 33 mg., with even higher levels in exceptional cases.

The experiments reported in this paper were initiated in 1934 to determine the endocrine basis for this remarkable rise in blood calcium. Realizing that the rise occurs while egg yolk is rapidly accumulating in the follicles, and following the lead of Russell, Howard and Hess (2) who showed that the serum calcium rose when an ovum attained a diameter of one centimeter or more, the working hypothesis upon which the investigations were based was that something accumulates in the yolk which, upon reaching a certain amount, has the ability to activate the parathyroids and thus to raise the blood calcium.

Evidence that egg yolk contains estrogenic hormone was found by Fellner (3), who tested a lipoid fraction from egg yolk on rabbits, and by Kopec and Greenwood (4), who were able to feminize growing feathers of a poularde by injecting egg yolk. On the other hand, Allen et al. (5) and Glimm and Wadehn (6) were unable to detect any evidence of sex hormones in yolk or in extracts of whole egg.

METHODS AND MATERIALS[1]

Procedures utilized included *a)* injection of egg yolk into immature females and capons to simulate the laying condition, *b)* injection of purified endocrine preparations and *c)* removal of egg yolk from laying fowls to simulate in part the non-laying condition.

Blood samples were drawn from the vena brachialis and tested for calcium content by the micro-determination method of Shapiro and Zwarenstein (7). According to these authors determinations on the same sera by the original Kramer-Tisdall method and by their modification of it did not differ by more than 0.4 mg. per 100 cc. of serum. Considering the levels of calcium studied in this paper such a small deviation is insignificant. All calcium determinations were made in duplicate.

[1] Grateful acknowledgment is made to the Schering Corporation for their kindness in furnishing progynon-B, to Ayerst, McKenna & Harrison, Ltd., for thyrotropic factor and to Parke, Davis & Co. for the extract of anterior pituitary used and for theelin.

Blood calcium was determined in all experimental birds before treatment, so that each bird served to some extent as its own control. In addition, untreated controls of the same breed and age as the experimental birds were kept in the same pen under identical conditions of management, feeding, light and temperature. All received an adequate diet containing ample cod liver oil.

EFFECTS OF EGG YOLK

Experiment 1. Immature females. Measured quantities of fresh egg yolk were shaken in a sterile container with a little Ringer's solution and injected into the peritoneal cavity of 6 pullets, which at the beginning of the experiment were 63 days old. The dose was 60 cc. of yolk per bird, distributed in 9 injections over 17 days.

TABLE 1. EFFECT OF INJECTION OF EGG YOLK UPON SERUM CALCIUM OF IMMATURE PULLETS 63 DAYS OLD

Group	No. of birds	Average mg. Ca per 100 cc. serum					Change[1] %
		Before test	Days after first injection				
			2	8	14	20	
Injected	6	14.3	16.3	19.2	19.3	14.7	+35.0
Control	6	—	16.6	16.7	15.6	15.1	− 6.02

[1] In tables 1 to 5 the last column gives the maximum deviation from the level before test; control reading on the same day.

Blood calcium of the yolk-injected birds rose steadily and at 8 days after the first injection was 34 per cent higher, at 2 weeks 35 per cent higher, than before injections were begun (table 1). Controls varied only slightly. The decline in blood calcium on the twentieth day is attributed to disturbance of the normal physiology by the mass of injected material. Although the rise induced is highly significant, the maximum level of 19.3 mg. does not equal

TABLE 2. EFFECT OF INJECTION OF EGG YOLK UPON THE BLOOD CALCIUM OF CAPONS

Group	No. of birds	Average mg. Ca per 100 cc. serum							Change %
		Before test	Days after first injection						
			2	5	8	11	14	19	
Injected	3	12.0	15.7	16.8	13.4	13.7	14.2	13.2	+40.0
Control	3	12.1	—	13.1	—	—	13.4	—	+ 8.3

the normal level for laying hens, which would ordinarily lie between 22 and 30 mg. This discrepancy is not surprising since in the normal ripening oöcyte the yolk material is surrounded by a follicle which is very highly vascularized. Any hormones in such yolk material would presumably be much more available and more effective than those in the injected yolk which lacked direct vascular connection.

Experiment 2. Capons. In order to have birds which could take, without ill effects, more yolk than could the immature Leghorn pullets, Barred Rock capons one year of age were used. Each received 100 cc. of yolk intraperitoneally over a period of 19 days. The results (table 2) show an increase of 40 per

cent to a level of 16.8 mg. on the fifth day, with somewhat lower readings thereafter. It is possible that the rather quick relapse of the blood calcium curve and the resumption of normal levels of calcium while injections were still being made in this and in other experiments resulted from the formation of antihormones or antibodies which counteracted the effects of the agent injected.

Experiment 3. Immature, but older, females. A repetition of this experiment, using New Hampshire pullets 100 days of age, yielded similar results

TABLE 3. EFFECT OF INJECTION OF EGG YOLK UPON THE BLOOD CALCIUM OF IMMATURE NEW HAMPSHIRE PULLETS 100 DAYS OLD

Group	No. of birds	Mg. Ca. per 100 cc. serum					Change %
		Before test	Days after first injection				
			12	16	18	23	
Injected	4	10.5	15.0	14.2	14.8	15.1	+43.8
Control	2	10.5	12.4	12.2	13.0	13.0	+23.8

(table 3). Following injection of 50 cc. of yolk per bird over 8 days, there occurred a rise of 43 per cent from 10.5 mg. before the test to 15 mg. on the twelfth day with a slightly higher level as late as the twenty-third day. In these birds the higher levels induced by treatment were maintained over a longer period than in the previous experiments and were consistently higher than those in the controls.

EFFECTS OF ESTROGENIC HORMONE

The assumption that the agent in the egg yolk responsible for the rise in blood calcium was of the nature of the estrogenic hormone led to experiments with two different preparations of that substance.

Estradiol monobenzoate. Three immature White Leghorn females, 96 days

TABLE 4. EFFECT OF INJECTION OF PROGYNON-B ON BLOOD CALCIUM OF IMMATURE WHITE LEGHORN PULLETS 96 DAYS OLD

Group	No. of birds	Mg. Ca per 100 cc. serum				Change %
		Before test	Days after first injection			
			3	7	11	
Injected	3	15.2	19.7	15.2	14.8	+29.6
Control	3	15.8	15.6	—	15.3	− 1.3

of age, were each given intramuscularly 500 R.U. of this substance in oil (progynon-B) over a period of 9 days (table 4).

By the third day after the first injection the average blood-calcium of these 3 birds was 19.7 mg., a figure over 29 per cent higher than the level before treatment. One bird showed an increase to 26.5 mg. Ca, a level that would be normal for a bird in full laying condition. Controls showed no change and in determinations at 7 and 11 days the experimental birds had lapsed back to the level of calcium prevailing before treatment. Effects of the injected

hormone did not last as long in this case as when yolk was injected.

Estrone (theelin). Intramuscular injection of 1400 R.U. of this substance in oil solution per bird over a period of 14 days resulted in an increase of blood calcium on the sixteenth day to 16.8 mg., a level over 64 per cent higher than in the same birds prior to the test.

Since both estrone and estradiol are able to raise blood calcium by significant amounts and since it would be quite natural for some form of the estrogenic hormone to be accumulated in egg yolk, it seems reasonable to conclude that the principle in egg yolk responsible for raising blood calcium is some form of the female sex hormone.

EFFECTS OF ANTERIOR PITUITARY

It has been suggested by some investigators that the anterior pituitary produces a parathyrotropic hormone. Since such a substance is more likely to be found in an extract of the whole gland than in any preparation of specific principles of that gland an extract of whole anterior lobe prepared by Parke, Davis & Company was first tried. When injected intramuscularly into immature White Leghorn females 150 days old at the rate of 4 to 6 cc. per bird over a period of 4 days, this substance was found to have no effect whatever on blood calcium.

Intramuscular injection of the thyrotropic fraction of the anterior lobe (8) in White Leghorn females 150 days old was followed in 5 days by a drop of 31 per cent in the level of blood calcium to 7.63 mg. per 100 cc. of serum. The depressing effect of the thyrotropic principle of the pituitary upon the level of blood calcium observed in these experiments is in agreement with the evidence of Prawochenski and Slizynski (9) that in the fowl there is an antagonistic action between the thyroid and parathyroid glands.

Since in some cases the synergistic action of two endocrine secretions differs from that of either alone, a trial was made to determine the effect of extract of anterior pituitary administered in conjunction with egg yolk. Three White Leghorn females, 150 days old, but not yet in laying condition, were given 4 to 5 cc. of anterior pituitary extract and 30 cc. of egg yolk over a period of 3 days. Serum calcium in these birds and their controls was unusually low before the test began, averaging 8.8 mg. for the experimental birds and 9.1 mg. in controls. The treatment induced a rise of 32 per cent to 11.7 mg. on the second day following the first dosage. In 2 birds which received 5 cc. of the extract, there was an increase of blood calcium to 14.4 mg. per 100 cc. of serum, a rise of over 65 per cent. At the same time the level in controls remained at 10.5 mg.

It is questionable whether the yolk alone could have been the sole cause of the rise, since in two previous experiments (albeit with younger birds) doses of 50 and 60 cc. of yolk alone were unable to induce a rise of more than 35 to 40 per cent. It is possible that the substance affecting blood calcium is more potent in birds 150 days old, and not far from laying, than in younger birds of 100 days or less. Otherwise this experiment would suggest that there is a synergistic action of some fraction of the anterior pituitary and of the principle in egg yolk.

REMOVAL OF EGG YOLK

Following the experiments previously described, evidence of the relation between some principle in egg yolk and the level of blood calcium was sought in a different way. If the injection of egg yolk into non-laying fowls induces a rise in blood calcium, then, conversely, the removal of yolk from females in full-laying condition should be followed by a temporary lowering of blood calcium.

To test this hypothesis 8 fowls in full-laying condition and with levels of blood calcium varying from 23.1 to 34.4 mg. were separated into three different lots and under nembutal anaesthesia operated upon[2] as follows.

Group A, 3 birds: Yolk was removed from the largest follicles, a different amount in each bird.

Group B, 3 birds: The three largest follicles were ruptured and the contained yolk squeezed into the body cavity.

Group C, 2 birds (controls): The largest follicles were disturbed but not ruptured and no yolk was removed.

The results of these experiments (fig. 1) show conclusively that some

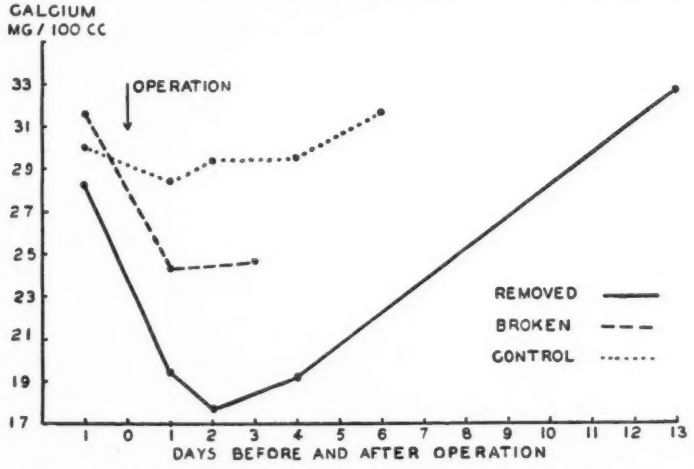


Fig. 1. The effects of removal of yolk and of rupturing mature follicles upon the serum calcium of laying females. The curves show averages for 3 birds in each experimental lot and for 2 in the controls.

substance in the egg yolk is instrumental in the maintenance of the high level of blood calcium characteristic of laying fowls.

Following removal of egg yolk the average blood calcium of the three birds dropped within 24 hours and on the second day was 17.7 mg., a figure 37 per cent lower than their average blood calcium (28.2 mg.) on the day before the operation. Furthermore, in these three birds the decrease was directly proportional to the amount of yolk removed (table 5). By the thirteenth day following the operation the average blood calcium of these three birds was up to 32.8 mg. and laying was resumed at 20, 15 and 16 days.

[2] The authors are indebted to Mr. R. K. Cole for conducting these operations.

Evidence that the lowering of blood calcium was not attributable to adverse effects of the operation is provided by the two control birds, *group C*. In these the level of calcium remained practically unchanged at 29.4 mg. on the second day after the operation and the birds continued to lay.

It is particularly interesting that the effect in *group B*, the birds in which

TABLE 5. EFFECTS ON BLOOD CALCIUM OF REMOVING DIFFERENT AMOUNTS OF YOLK

Bird no.	Yolk removed cc.	Serum Ca, mg. per 100 cc.		Decrease %
		Before operation	Two days after	
C-112	15	23.1	17.4	24.7
C-132	24	31.1	19.8	36.3
C-158	37	30.5	15.8	48.2
Average		28.2	17.7	37.2

yolk was squeezed out of the follicles but left in the body, was intermediate between the normal level maintained by the controls and the extreme reduction experienced in *group A*. The average for this group was 24.31 mg. on the day after the operation, a drop of 23 per cent from the level before the operation. This would indicate that although none of the yolk was removed from the body cavity, its influence on blood calcium was considerably reduced when the yolk was separated from the highly vascularized follicles through which it is ordinarily effective. The condition of these birds was very similar to that of the birds in which yolk was injected. It will be recalled that while all of these experienced a rise in blood calcium in none was the normal calcium level of laying hens attained.

DISCUSSION

After most of these experiments had been concluded it was reported by Riddle and Dotti (10) that significant increases in the blood calcium of pigeons, doves, fowls, rats and dogs were induced by injections of theelin and progynon-B. The recent work of Levin and Smith (11) indicates that estrogens have very little effect, if any, upon the serum calcium of mammals, but there can be little doubt that these agencies have an extremely important influence upon the level of calcium in birds. The failure of Marlow and Koch (12) to find any consistent rise in the blood calcium of their fowls treated with female sex hormones may seem at first sight to conflict with the results presented in this paper, and with those of Riddle and Dotti, but it is evident that the effects of their treatments were obscured by great variations in the blood calcium of their controls and experimental animals. Such variations are inevitable in mature fowls and in those close to laying age. They were reduced to a minimum in the present experiments by the use of birds less than 150 days of age. It is true that the pre-test levels of calcium varied from lot to lot, possibly because the different lots were hatched (and used) at different seasons of the year and following different degrees of confinement. In any one experiment controls and experimental birds differed only very slightly at the beginning of the test.

The object of the present experiments was not merely to find some endocrine preparation which in large doses might affect calcium levels, but rather

to determine how the rise occurs in the fowl under natural conditions. To complete the chain of evidence it was necessary to determine if the estrogenic hormone in the yolk acts directly on the blood calcium or indirectly by stimulating the parathyroids. From histological studies (to be presented elsewhere) of the latter glands in non-laying, laying and yolk-injected fowls it seems certain that the estrogen in the yolk affects the parathyroids, either directly or indirectly, and that their activation results in the higher level of serum calcium characteristic of laying fowls.

This permits reconstruction in part of the major endocrine reactions associated with laying. Presumably at maturity the gonadotropic hormone of the anterior pituitary is produced in greater quantity, thus inducing deposition of yolk in the oöcytes. (This reaction can be accelerated even before maturity by stimulation of the pituitary with artificial light.) Accumulation of yolk is accompanied by accumulation of the estrogenic hormone produced by the ovary and when an adequate amount is available the parathyroids are stimulated to action.

The failure of an extract of anterior pituitary to affect blood calcium in the present experiments may seem in contrast to the results of Shapiro and Zwarenstein (7) and of Friedgood (13). However, the positive effects of these investigators were obtained in amphibia and in mammals, not in birds. As Loeb, Anderson, et al. (14) have shown, species differ in the activities of their anterior pituitaries. The contrasting effects of prolactin in mammals and in birds show that species can also differ in their response to certain hormones.

SUMMARY

Injection of 50 to 100 cc. of egg yolk into the peritoneal cavities of immature female fowls and of capons induced a significant rise in the levels of blood calcium. Similar results were obtained by the use of the estrogenic hormone in the form of progynon-B and as theelin. Removal of 15 to 37 cc. of yolk from laying hens caused a significant drop in the level of blood calcium, the decrease varying from 25 to 48 per cent and being directly proportional in different birds to the amount of yolk removed. Operated controls were unaffected but in birds in which yolk was squeezed from the follicles and left in the body cavity the serum calcium dropped 23 per cent. The thyrotropic principle of the anterior pituitary depressed blood calcium and an extract of whole anterior pituitary had no effect.

REFERENCES

1. Riddle, O., and W. H. Reinhart: Am. J. Physiol. **76**:660. 1926.
2. Russell, W. C., C. H. Howard and A. F. Hess: Science **72**:506. 1930.
3. Fellner, O. O.: Klin. Wchnschr. **4**:1651. 1925.
4. Kopec, St., and A. W. Greenwood: Arch. f. Entwlngsmechn. d. Organ. **121**:87. 1929.
5. Allen, J., J. W. Whittsett, J. Hardy and F. L. Kneibert: Proc. Soc. Exp. Biol. & Med. **21**:500. 1924.
6. Glimm, E., and F. Wadehn: Biochem. Ztschr. **179**:3. 1926.
7. Shapiro, H. A., and H. Zwarenstein: J. Exper. Biol. **10**:186. 1933.
8. Anderson, E. M., and J. B. Collip: Proc. Soc. Exp. Biol. & Med. **30**:680. 1933.
9. Prawochenski, R., and B. Slizynski: Biol. generalis **12**:498. 1936.
10. Riddle, O., and L. B. Dotti: Science **84**:557. 1936.
11. Levin, L., and P. E. Smith: Endocrinology **22**:315. 1938.
12. Marlow, H. W., and F. C. Koch: Endocrinology **21**:72. 1937.
13. Friedgood, H. B.: Endocrinology **20**:159. 1936.
14. Loeb, L., W. C. Anderson, J. Saxton, S. J. Hayward, A. A. Kippen: Science **82**:331. 1935.

CLINICAL AND LABORATORY NOTES

PROTAMINE INSULIN

THREE PRACTICAL POINTS

G. B. ROBSON AND H. GRAY*

The clinical aspects of Hagedorn's protamine insulin were demonstrated by Krarup in 1935 clearly and in detail. In the succeeding 3 years there have appeared 47 articles which can easily be traced in the *Quarterly Cumulative Index Medicus*. Much space has been given to confirmatory evidence and experienced diabeticians around the world have added refinements. There remain three features, however, which are worthy of remark, the occurrence of lumps and of reactions, and the use of large dosage.

Forty of our records yielded suitable information. The diet averaged 135 gm. of carbohydrate, 72 gm. of protein, 84 gm. of fat and 1500 calories. The total insulin per 24 hours averaged 45 U. It was given *a)* partly as protamine (or more recently protamine-zinc) insulin and partly as regular insulin, in 7 cases, *b)* all as protamine insulin in 33 cases. As experience has increased we find ourselves encouraging patients more and more to use protamine insulin alone.

Dislike of protamine is, however, to be reckoned with. The proportion of patients on protamine insulin who quit taking it, at least for a while, was small in Joslin's series, only 3 per cent of 1250 cases, but was large in the present series, i.e., 9 of 40 cases. Joslin's fewer defections are attributable to his treatment of patients in private office and hospital, while ours are treated in an out-patient clinic. The reasons given for abandonment by our 9 patients were as follows.

a). No reason, or ignorance, in three.

b). Lumps, in two.

c). Itching, in one, enough to interfere with sleep.

d). Nausea, without sweating or other usual hypoglycemic signs, in one. This phenomenon, like morning headache without other symptoms, is peculiar to protamine insulin and fairly common.

e). Neuritis of shoulder, without reaction, in one. (Joslin speaks of "severe neurological symptoms following reaction.")

f). Preference for low-carbohydrate diet without insulin, in one. In this connection it is of some interest that even with protamine insulin the diets reported by Krarup for 37 protocols averaged 125 gm. C, 72 P, 137 F, and 2021 calories.

Lumps. Subcutaneous nodules, measuring usually 1 to 2 or even 5 cm. in diameter, appeared in about 1 in 10 patients. These developed within 1 to 3 hours, were moderately red and tender, itching occasionally, lasted 3 to 7 days, and were non-suppurating.

Twenty or exactly half of our patients had lumps after protamine, none after regular insulin. On writing three manufacturers we learned that nodules are rare

* From the Stanford University School of Medicine, San Francisco, Calif.

and unexplained. Similarly in the literature little was found. Rudy in discussion of Joslin's paper reported one patient who had nodules which cleared up so slowly that on one day he counted as many as 16, and Jordan wrote briefly of "local reactions with redness, swelling, tenderness, pain, prolonged induration."

Five patients had lumps from only one manufacturer's brand of the three, but not all were sensitive to the same brand. Eight were sensitive to two brands, three to all, while the remaining four were sensitive to one brand but were not tried on the others. We conclude that the three brands are of equal merit on the whole, though for a given individual one is often preferable.

On an hypothesis of allergy we injected 0.1 cc. intracutaneously, 40 U strength, protamine in one arm, regular in the other, and examined 20 minutes later for erythema, wheals or pseudopods. In 8 patients the results were: only one slightly allergic to protamine (Mfr. C), but 7 slightly reactive to regular insulin (1 Mfr. A, 4 Mfr. B, 2 Mfr. C). We conclude that allergy as a factor in lumps is negligible. With continued use the lumps became definitely diminished in one patient and somewhat diminished in two. One patient discovered for herself that injection in the abdominal wall obviated lumps.

Since we routinely prescribe ¼-inch needles because less breakable, the hypothesis suggests itself that the lumps may be due to injection into the subcutaneous fat rather than beneath it. As a test we furnished 1-inch needles to 7 patients, insisting that after insertion under the skin it should feel free as if inside a tennis ball not as if in an orange. Four patients were relieved promptly of their lumps, three were not. We conclude that this factor, like the others discussed above, must be remembered, but is variable.

Reactions. This subject is "unduly minimized in many of the reports," as insisted by Wilder in 1937.

Evidence in print is scanty. Jordan recorded unconsciousness in 35 per cent of his series, Drysdale observed reactions in 40 per cent of juvenile diabetics. In our series of 40, though it included only 4 children and 3 adults with child-like brittle blood-sugars, at least 25 patients, i.e., 62 per cent, reported hypoglycemic reactions. Of these 25, 12 had their reactions rather frequently or severely or both; but no accidents. We believe that some chances must be taken in order to secure sugar-free urine tests, even though we do not insist on constant sugar freedom. The 62 per cent just reported, high as it is, we believe would be increased, and truly, by careful questioning. Reactions will be overlooked unless specifically asked for, because many patients, especially in the hard-working non-neurotic outpatient group, either do not particularly notice moderate symptoms, or accept them without ado.

We believe the practice is too common of dodging reactions by permitting patients to run along with tests as bad as yellow-green or yellow.

The symptoms of reaction, though so often listed, are not yet well known, and perhaps cannot be precised in any individual. Among them the insufficiently emphasized are headache on waking (Joslin, Mosenthal, Rudy), less often nausea.

Bedtime food has served us extremely well as a preventive. Hypoglycemic shock is unquestionably reduced by this carbohydrate at bedtime, and rarely requires multiple meals. The benefit of food-fractionation in flattening the blood-sugar-curve is sometimes called the Staub-Traugott effect. Priority is really due to Hamman and Hirschman 1919, if not indeed to earlier authors, as pointed out by one of us in 1922.

Large Dosage. Insulin, like thyroid, is more frequently given in too small than too big amounts. It should be pushed to tolerance and entails no unreasonable risk if increased gradually. Ordinarily we advise patients to make the Benedict test before

breakfast every fourth day, if sugar-free to decrease the protamine 4 units, if green to make no change, if yellow to increase 4 units. Some patients in the present series have, with no ill effect, gradually increased their protamine to doses which we find cause surprise to sundry observers, hence the present comment. Our largest doses at the moment are as follows. *a)* In terms of total insulin per 24 hours: 88, 96, 136, 140, 144 and 160 U. These are equalled or exceeded by the reported doses of 100 U (Drysdale 1937), 132 U (Wilder 1937), 140 U (Rabinowitsch 1936), 400 U (Joslin 1938). *b)* In terms of protamine at any one dose: 70, 70, 80, 80, 90 and 100 U. Reached by the gradual 'four day-four units' method, large doses cause the patients no trouble and us no loss of sleep.

We wish to express our gratitude to the manufacturers for liberal supplies of insulin (Eli Lilly and Company through Dr. F. B. Peck, Sharp and Dohme through Dr. G. H. Krall, and E. R. Squibb and Sons through Dr. H. S. Newcomer), and to an anonymous donor for defraying other expenses.

THE TREATMENT OF ACNE VULGARIS WITH TESTOSTERONE PROPIONATE*

MATTHEW MOLITCH

Following the report of Hamilton[1] of improvement of acne vulgaris in a patient after the use of testosterone, we decided to determine the value of testosterone propionate under controlled conditions. Twenty-two otherwise healthy young white men, ranging in age between 17 and 23 years, were selected for this study. The skin lesions varied from the mildest to the most severe forms. All of the subjects received 3 intramuscular injections† a week without any local treatment, internal medication, or change of diet. Twelve of these patients received injections of the testosterone in sesame oil, whereas the remainder were given only sesame oil. Both groups showed a variety of the different types and degrees of acne vulgaris. A dermatologist examined the patients before and after treatment without knowledge as to which ones were in the control or the treated groups.

TABLE 1. CONTROL GROUP

Case	Age	No. of Injections	Results	
			Objective	Subjective
D. F.	20	23	25% improved	improved and skin less oily
S. J. L.	18	22	no improvement	slightly improved
F. H.	17	23	25% improved	slightly improved
G. N. H.	18	23	no improvement	skin less oily
S. B. M.	18	23	no improvement	improved
G. M.	17	23	25% improved	improved
G. L.	18	23	slightly improved	slightly improved
C. G.	19	22	no improvement	slightly improved
H. R.	17	23	50% improved	improved
P. R.	18	21	50% improved	improved and skin less oily

No local or systemic reactions occurred in either group. No cases of priapism occurred, nor was the incidence of nocturnal emissions or erections increased. In the tables it will be noted that the control group received fewer injections than the treated group. This was because we felt that additional information would not be obtained by continuing the control group beyond the second month. The treated group was given 3 injections a week for 3 months; the dose for the first month was 5 mg., for the second month 10 mg., and for the third month 25 mg. The control group received 1 cc. of sesame oil at each injection. Table 1 gives the details and results of the control group, table 2 gives the details and results of the treated group, and table 3 gives a comparison of both groups. It will be noted that about the same degree of improvement occurred in both groups.

Deanesly and Parkes[2] showed that the propionic ester of testosterone is ab-

* From the School of Medicine, University of Pennsylvania, Philadelphia, Pa.

† We wish to thank Dr. M. Gilbert of the Schering Corporation for supplying the testosterone propionate used in this study. We also wish to thank Dr. R. L. Gilman for examining the subjects dermatologically.

[1] Hamilton, J. B.: Endocrinology 21:649. 1937.

[2] Deanesly, R., and A. S. Parkes: Proc. Roy. Soc. London, s, B. 124:279. 1937.

sorbed through the skin. We treated 7 young men with acne, using an ointment containing 50 mg. per 30 gm. About 4 gm. was used nightly over a period of 3 months with the same degree of improvement as was noted in our injected groups.

Summary. Twelve young men with various types and degrees of acne vulgaris were treated with intramuscular injections of testosterone propionate over a period of 3 months. Ten similar patients were given injections of a bland oil and served as

TABLE 2. TREATED GROUP

Case	Age	No. of Injections	Testosterone Total Doses (mg.)				Results	
			mg.	5	10	25	Objective	Subjective
A. J.	20	33	405	12	12	9	50% improved	slightly improved
E. H.	19	35	455	12	12	11	35% improved less oily	slightly improved
C. A. E.	17	25	310	9	9	7	less cystic otherwise no improvement	improved
G. M.	18	30	360	12	10	8	50% improved	improved and skin less oily
C. M. J.	18	34	430	12	12	10	improved	improved and skin less oily
H. T. W.	21	32	395	12	11	9	no improvement	slightly improved
Y. L.	16	32	380	12	12	8	no improvement	cyclic improvement
K. S.	17	32	380	12	12	8	no improvement	improved and skin less oily
S. R. W.	23	31	375	11	12	8	slightly improved	slightly improved
P. A. G.	17	31	375	11	12	8	no improvement	improved
L. J. W.	19	14	260	—	6	8	no improvement	no improvement
G. J.	18	27	255	12	12	3	slightly improved	slightly improved and skin less oily

TABLE 3. COMPARISON OF RESULTS

Group	No. of Cases	Improvement			
		Objective		Subjective	
		No.	%	No.	%
Control	10	6	60.0	9	90.0
Treated	12	6	50.0	10	83.3

controls. The degree of improvement, both subjective and objective, was about equal in both groups. Seven additional patients were treated locally with an ointment containing testosterone propionate with similar results. It is possible that acne vulgaris may be relieved by this product if an associated hypogonadism is present. The high percentage of improvement obtained in both groups suggests that a marked psychogenic element was present in our cases. No increase in the incidence of nocturnal emissions or erections was noted.

705 Pacific Avenue, Atlantic City, N.J.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

HYPOPHYSIS

The endogenous nitrogen and basal energy metabolism relationships in hypophysectomized rats. Ashworth, U. S., and G. R. Cowgill, Am. J. Physiol. **122**:373. 1938.

Studies made on 19 hypophysectomized rats showed that the basal heat production as determined by the Haldane gravimetric method was 57% less than that of normal controls. The endogenous nitrogen metabolism as judged by the nitrogen excretion on low protein and protein free diets was unchanged in the operated animals, although they excreted considerably less nitrogen during the first 5 or 6 days on the diet. This fact was interpreted as indicating that hypophysectomized animals have less stored protein than normal controls. It is concluded that there is no constant relationship between the minimum nitrogen requirement and basal metabolism.—F. A. Hitchcock.

The heart in acromegaly. Courville, C., and V. R. Mason, Arch. Int. Med. **61**:704. 1938.

This report is based on the observations of 24 patients with acromegaly. Of this group, 18 (75%) presented evidence of marked heart failure, and 6 have died of heart failure. These 6 patients all had marked splanchnomegaly and cardiomegaly, and an eosinophilic pituitary adenoma was observed post-mortem.—I. B.

Influence of lactation on the gonadotropic function of the anterior lobe of the hypophysis in white rats (Influence de la lactation sur les fonctions gonadotropes du lobe antérieur de l'hypophyse chez le rat blanc). Desclin, L., and Ch. Grégoire, Compt. rend. Soc. de biol. **126**:250. 1937.

Twenty white rats were spayed 2-3 days after the birth of their litters. Half were deprived of their litters and half were allowed to suckle. Ovaries from 10 rats, 25-35 days old, were removed and one of each pair was transplanted to the kidney of a suckling spayed rat, while its partner was placed in a non-suckling spayed animal. In the suckling animals the ovarian graft showed, after 2 weeks, no corpora lutea and no ripe follicles. In the non-suckling animals the grafts were much enlarged and contained numerous corpora lutea. These results support Riddle's observations that prolactin acts antagonistically to the follicle-stimulating hormone.—J. C. D.

The ultimobranchial body (Der ultimobranchiale Körper). Eggert, B., Endokrinologie **20**:1. 1938.

Histological and experimental studies made on the wall lizard indicate that the ultimobranchial body constitutes an endocrine organ which undergoes regression, the cause of which is unknown. Its activity is stimulated by the thyrotropic hormone of the anterior hypophysis.—W. J. A.

Simmonds's disease (hypophyseal cachexia). Escamilla, R. F., and H. Lisser, California and West. Med. **48**:443. 1938.

Nine cases were presented in which the history and findings were suggestive of Sim-

monds's disease. The classical and most constant findings are loss of weight, loss of sexual function, and lowered B.M.R. The authors feel that this disease should be suspected when a patient presents continued loss of weight for which no other cause can be found. Clinical differentiation from anorexia nervosa is occasionally very difficult, and the subsequent course of one case caused it to be placed in this category. Treatment is discussed and, although therapy by replacement with APE seems logical, its administration has thus far, at least in the experience of these authors, been somewhat disappointing. More potent preparations are needed. Despite the low B.M.R. found in these patients, administration of thyroid substance is ineffectual. A fattening regimen with the use of insulin may be tried cautiously. The condition of some patients may improve spontaneously, but the eventual prognosis is usually grave.—I. B.

Dystocia in diabetes insipidus. The relation of pituitary oxytocin to parturition. Fisher, C., H. W. Magoun and S. W. Ranson, Am. J. Obst. & Gynec. **36**:1. 1938.

In 7 cats diabetes insipidus was produced by appropriate hypothalamic lesions during various stages of pregnancy. They were partially or totally unable to deliver the young, or succeeded only after labor greatly prolonged. Four died in labor, 2 shortly after, 1 survived. It is concluded that work so far reported does not warrant rejection of pituitary oxytocin as a factor in normal parturition.—E. L. S.

An evaluation of the anterior pituitary-like substance intradermal test for pregnancy. Friedman, J. J., and H. Fink, Am. J. Obst. & Gynec. **36**:116. 1938.

Tests with known pregnant and non-pregnant women, and with men showed the technic to be unreliable as shown by other critical studies.—E. L. S.

Plasma proteins in pituitary insufficiency (Acción de la insuficiencia hipofisaria sobre las proteínas del plasma). Goldberg, I., Rev. Soc. argent. de biol. **14**:49. 1938.

Hypophysectomy was followed in dogs by a slight increase in total plasma proteins; this was due to an increase in globulins, albumin decreased slightly. The A:G ratio decreased. Injection of APE produced an increase in plasma proteins in normal, thyroidectomized and hypophysectomized dogs. After prolonged thyroid treatment the plasma proteins returned to normal. These results, together with the fact that thyroidectomy produces similar alterations in the plasma proteins to those seen in hypophysectomized dogs, makes it plausible to attribute them to thyroid insufficiency induced by removal of the pituitary. In toads a decrease in plasma proteins was observed after hypophysectomy; the A:G ratio increased.—J. T. L.

Determination of the ketogenic activity of extracts of endocrine organs. Gray, C. H., Biochem. J. **32**:743. 1938.

When crude saline or thyrotropic extracts of anterior pituitary are injected into fasting female rats, there is an increase in liver fat followed by increased excretion of acetone bodies. In male rats ketonuria is greater but liver fat is less affected. Cortin (Organon) did not increase ketonuria in doses up to 3 cc.—D. L. T.

Work-performance of hypophysectomized rats treated with cortin. Ingle, D.J., Am. J. Physiol. **122**:302. 1938.

The work-capacities of the gastrocnemius muscles of 45 hypophysectomized rats treated with cortin were compared with those of 45 untreated hypophysectomized rats. Immediately after operation there was but little difference in the work-performance of the treated and untreated animals. After periods of delay of 4 and 7 days following operation the initial rates of work and the work-totals of the cortin treated animals were significantly higher than those of the untreated animals. The work-performance of the hypophysectomized rats, even when treated with cortin, was far below the standard for normal animals.—C. Duncan.

The differential migration of the pressor and oxytocic hormones in electrophoretic studies of the untreated press-juice of the posterior lobe of the pituitary gland. Irving, G. W., and V. du Vigneaud, J. Biol. Chem. **123**:485. 1938.

In the electrophoretic study of the chemically untreated, mechanically expressed juice of fresh posterior pituitary glands, it was found that the pressor activity travelled at a faster rate than the oxytocic activity, thus demonstrating that the activities in this simple press-juice were manifestations of different chemical entities.—J. M. L.

The treatment of late pubertal emaciation with hypophyseal transplantation (Die Behandlung der spätpubertären Magersucht mit Hypophysentransplantation). Kylin, E., Acta med. Scandinav. **96**:75. 1938.

In 31 cases of postpubertal emaciation occurring in young women transplantation of calves' hypophyses were made. In 20 cases the women were able to return to work, and in 2 others there was improvement although the patients were not able to work. In 13 cases menstruation recurred after operation. Two patients died and 4 showed no improvement.—J. M. L.

The effects of posterior pituitary on isolated intestinal segments. Larson, E., J. Pharmacol. & Exper. Therap. **63**:20. 1938.

All segments of dog intestine gave unsatisfactory responses but the guinea-pig or cat iliac or colonic and rabbit jejunal segments were satisfactory. No differences could be detected in the responses to pitressin, posterior pituitary or depressor-free pituitary. Pitocin gave no response.—E. L.

The hypophysis in total deviation of the bile to the outside (La ipofisis nella derivazione totale della bile all'estero). Lauro, A., Sperimentale, Arch. di biol. **92**:28. 1938.

Deviation of bile causes the development of lesions in the pituitary, especially in the anterior lobe, which become more accentuated with time of survival. Treatment with pituitary extract seems to have some beneficial influence.—A. E. M.

The effects of diets low in choline upon liver function, growth and distribution of fat in the white rat. McLean, D. L., J. H. Ridout and C. H. Best, Brit. J. Exper. Path. **18**: 345. 1937.

Experiments were carried out to study further the effects of diets low in choline and other lipotropic factors on liver function, on the rate of increase in weight, and on the distribution of fat in the white rat. The choline-fed animals gained weight, whereas the controls receiving the same diet without choline lost weight. Tests to detect loss of liver function were positive in a certain number of animals with very fatty livers, while they were uniformly negative in choline-fed animals. Studies in rats with fatty livers indicated that these animals excrete during starvation appreciably larger amounts of ketone bodies than normal rats (confirming Deuel and collaborators), but that the extent of the ketonuria is not proportional to the amount of excess fat present in the liver. Large amounts of fat may be present in the liver after the ketonuria has largely subsided and a further excretion of ketone bodies may then be elicited by the injection of certain extracts of the anterior pituitary gland.—Margaret Baptist.

The influence of anterior pituitary extracts, injected either with or without insulin, on the glycogen contained in the livers of fasting young rabbits. Marks, H. P., and F. G. Marks, J. Physiol. **93**:61. 1938.

The rise in the liver glycogen which usually follows the administration of insulin to fasting young rabbits was prevented by the injection of prolactin or thyrotropic fractions into 16 rabbits, but not by crude saline pituitary extracts (8 rabbits). This active factor may be identical with the glycostatic of Russell; but different from Young's glycotropic, prolactin, gonadotropic and thyrotropic. There was also a great increase in the weight of the liver following administration of all 3 extracts; due to the presence of extra total water and fat as well as glycogen.—J. W. Jailer.

Integrative interaction of hypophysis, thymus and ovary (Untersuchungen über das integrative Zusammenwirken von Hypophysen-, Thymus-, und Ovarialhormon). Müller, C., Endokrinologie **19**:289. 1937.

Using young castrated female albino rats, the influence of thymectomy and of hypophysectomy on the estrogenic effect of injected follicular hormone was determined. While thymectomy was without any effect, hypophysectomy increased the activity of a given dose at least 6 times, and in some cases as much as 20 times. It is concluded that the hypophysis exerts a specific influence in raising the threshold of stimulation of the genital tract.—W. J. A.

Interaction of hypophyseal and ovarian hormones (Untersuchungen über das Zusammenwirken von Hypophysen- und Ovarialhormon). Müller, C., Endokrinologie **20**:8. 1938.

Using castrated and castrated hypophysectomized female rats, the influence of thyroidec-

tomy upon the estrogenic effect of Östroradiolbenzoat, as shown by the Allen-Doisy test, was determined. While hypophysectomy increased the effect, thyroidectomy was without effect upon the onset of estrus.—W. J. A.

The anatomy and pharmacology of the hypophysis of the armadillo. Oldham, Frances K., J. Pharmacol. & Exper. Therap. **63**:31. 1938.

The posterior and anterior lobes are separated completely by a septum and the intermediate lobe is absent in the armadillo. The oxytocic, pressor and anti-diuretic activities are in the posterior lobe with the melanophore-expanding activity present in the anterior lobe. The glandular cells present may elaborate the posterior lobe hormones.—E. L.

Lactogen content of pituitary glands from rats on vitamin deficient rations. Reece, R. P., C. W. Turner, I. L. Hathaway and H. P. Davis, Proc. Soc. Exper. Biol. & Med. **37**:293. 1937.

Litter-mate rats were divided into two groups, one of which received a diet deficient in either vitamin A, B, D, or E. The other group of litter mates served as controls. The lactogen content was determined by the local crop sac method, the experimental pituitary being implanted on one side, and the control pituitary being implanted on the opposite side of the test animal after suitable maceration. As compared with their litter-mate controls, the pituitaries of vitamin-A- B and D-deficient animals were somewhat lower in lactogen content. In the case of the vitamin-B-deficient animals this decreased content was observed only when the comparison was made on whole gland content, but not in content per milligram of pituitary tissue. On either basis, vitamin-E-deficiency gave no evidence of leading to decreased content.—M. H. F.

Pro-gonadotropic sera. Rowlands, I. W., Proc. Roy. Soc. London, s.B. **124**:492. 1938.

The serum of a sheep chronically injected with sheep pituitary extract did not develop antigonadotropic activity but increased the activity, on test rats, of gonadotropic extract from sheep glands; although the serum itself had no gonadotropic action. The serum of a goat chronically injected with pig pituitary extract likewise enhanced the action of pig, sheep, and ox extracts on ovarian weight in test rats; yet this same serum inhibited the gonadotropic effect of extracts of horse pituitary, pregnant mare serum, and human pregnancy urine, and inhibited the power of the pig pituitary extract to produce ovulation in estrous rabbits. It is suggested that such sera contain an antihormone or neutralizing substance to the 'pituitary antagonist' of Evans.—D. L. T.

The specificity of antigonadotropic sera. Rowlands, I. W., Proc. Roy. Soc. London, s.B. **124**:503. 1938.

Antigonadotropic sera were obtained by treating rabbits with preparations from ox pituitary, horse pituitary, pregnant mare serum, and human pregnancy urine. In the 2 last cases the antisera showed complete species-specificity, in the 2 first cases they did not; complete source-specificity was shown only by the antisera to pregnant mare serum.—D. L. T.

The effect of thyroxin on the carbohydrate metabolism of hypophysectomized rats. Russell, Jane A., Am. J. Physiol. **122**:547. 1938.

The intraperitoneal injection of thyroxin in young male hypophysectomized rats restored to normal the rate of absorption of glucose from the intestine but was without effect on the oxidation of glucose and the deposition of glycogen. The dose necessary to produce this effect was about half that necessary to restore the O_2 consumption to normal.—F. A. Hitchcock.

Recent advances in knowledge of the anterior lobe of the hypophysis. Rynearson, E. H., and C. H. Hodgson, Arch. Int. Med. **62**:160. 1938.

Most of the hormonal substances of value in clinical medicine have been obtained from the pancreas, thyroid gland, sex glands and adrenal glands, rather than from the anterior lobe of the hypophysis, which has an effect on all of them. A new era began when Evans and Long reported their work with the growth hormone in 1921. Since then thousands of articles have reported work on the many hormones, real or suspected, which are attributed to the anterior lobe of the hypophysis. Separate hormones have been reported to have an effect on growth; lactation; behavior; blood; pigmentation; metabolism of carbohydrate, protein, fat and water; production of ketone bodies, and activity of the thyroid, parathyroid, pancreas, adrenal and sex glands—and this list is incomplete. When incomplete knowledge of these effects reached the ears of some indiscriminating and overly enthusiastic clinicians, there followed the publication

of many more 'contributions' to medical literature. Within recent years a semblance of order has developed. There are few clear-cut syndromes resulting from disturbances of the anterior lobe of the hypophysis; these are dwarfism; acromegaly or gigantism; Froehlich's syndrome; Cushing's syndrome, and, rarely, Simmonds's disease (pituitary cachexia). Advance will continue along two lines: first, it will be made in the laboratories. Contributions and confusion are to be found in the laboratory study of the anterior lobe of the hypophysis. This work is of fundamental importance, and it must be encouraged in every institution in which it is in progress. Second, advance will come from the scientific application of this knowledge to patients. Splendid results have been achieved, and physicians should be encouraged to treat patients whom they can study carefully and observe closely. Progress is impeded by the continued improper publication of the results of unplanned and mismanaged treatment of patients who receive unidentified preparations for undiagnosed conditions.—I. B.

Pitocin in the third stage of labor. White, M. R., Am. J. Obst. & Gynec. **36**:90. 1938.

Intravenous or intramuscular pitocin produces no undesirable systemic effects. It is not contraindicated in toxemias. Intravenous pitocin at the beginning of the third stage of labor induces prompt, uniform uterine contraction, hastening separation and expulsion of placenta without uterine trauma or laceration of placenta or membranes. There is no increase in placental retention. Blood loss and duration of the third stage were lower with intravenous than with intramuscular pitocin. The duration of oxytocic action is shorter than that of ergotrate. The study is based on observations in 630 patients.—E. L. S.

The production of antisera to preparations of prolactin containing the glycotropic (anti-insulin) factor of the anterior pituitary gland. Young, F. G., Biochem. J. **32**:656. 1938.

A prolactin preparation, devoid of demonstrable thyrotropic activity, but capable of rendering rabbits refractory to insulin, was injected in large doses (equivalent to 1 gm. dried gland per day) into adult rabbits. After 18 weeks the rabbits had become insensitive to the glycotropic factor and responded normally to insulin; but injections of the serum of such rabbits did not make other animals resistant to the glycotropic factor. After 12 weeks, the serum rendered pigeons non-responsive to prolactin and guinea pigs non-responsive to thyrotropic extracts. Anti-prolactin sera were also produced in monkeys. The rate of growth of the young of lactating mice injected with anti-prolactin rabbit serum is slightly retarded.—D. L. T.

The diabetogenic action of crude anterior pituitary extracts. Studies on the fractionation of diabetogenic extracts of the anterior pituitary gland. Young, F. G., Biochem. J. **32**:513. 1938.

In most dogs, and in some cats and rabbits (but not in mice, rats, or guinea pigs), daily injection of a crude extract of anterior lobes (beef) produces glycosuria and ketonuria. In the dog, the effect is seen only after 3 or 4 days, and may disappear in 4 or 5 days in spite of continued treatment, unless the dose is increased; in some cases, after treatment with large doses, the diabetogenic effect persists long after treatment has stopped. The active principle becomes inactive if the extracts are not kept at low temperatures; inactive fractions may retain the power to produce polyuria in dogs. The diabetic state cannot be produced with large doses of a preparation of prolactin which contains the glycotropic or anti-insulin factor (rendering rabbits insensitive to insulin), nor with partly-purified thyrotropic fractions; but it can be produced with extracts from which prolactin has been removed. The term 'diabetogenic factor' should not be used in cases where only transient hyperglycemia or glycosuria are produced, without ketonuria and polyuria; the diabetogenic factor is probably complex.—D. L. T.

PANCREAS

The effect of thyroidectomy upon pancreatic diabetes in the cat. Dohan, F. C., and F. D. W. Lukens, Am. J. Physiol. **122**:367. 1938.

The urinary excretion of glucose, nitrogen and acetone bodies was determined in 6 depancreatized cats (Group *A*), 10 cats in which a piece of neck muscle was removed at the same time they were depancreatized (Group *B*), 6 cats that were thyroidectomized 4 to 34 days before pancreatectomy (Group *C*), and 13 cats depancreatized and thyroidectomized at the same time (Group *D*). Both group *C* and *D* showed a decrease over the controls (*A* and *B*) in the excretion of all 3 substances studied, but the decrease was statistically significant only with glucose and nitrogen in group *C* and with nitrogen in group *D*. It is concluded that the thyroid has only a slight influence on pancreatic diabetes.—F. A. Hitchcock.

Hypoglycemia. Hartmann, A. F., and J. C. Jaudon, J. Pediat. **11**:1. 1937.

An analysis has been made of all cases of hypoglycemia studied in the St. Louis Children's Hospital over a period of 15 years (286 cases). Pathogenesis and classification on an etiologic basis have been emphasized, together with data obtained particularly from 3 groups: *a)*, normal newborn infants; *b)* infants born of diabetic mothers, and *c)*, children developing recurring attacks of very severe hypoglycemia, who seem to be insulin hypersensitive or intolerant, chiefly because of lack of proper opposing secretions (adrenal and pituitary). The following conclusions have been reached. *a)* Hypoglycemia during the first 4 or 5 days of life occurs quite regularly in normal newborn infants, and seems due to an imperfectly developed regulatory mechanism which creates a state of relative hyperinsulinism; *b)*, A similar period of hypoglycemia with the development occasionally of such very severe manifestations as convulsions and collapse occurs in infants born of diabetic mothers. The greater fall in blood sugar in such infants (sometimes with a striking absence of symptoms) seems more often referable to an increased physiologic hyperactivity of the islands of Langerhans than to the development of islet cell hypertrophy or hyperplasia, and may be prevented or controlled quite effectually by the combined use of epinephrine and dextrose injection as emergency measures, and the prophylactic starting of complemental mild and carbohydrate feedings immediately after birth; *c)*, The natural tendency to the development of hypoglycemia in the first few days of life may be greatly intensified also by adrenal and intracranial hemorrhage; *d)*, The demonstration of intolerance or increased sensitivity to insulin in subjects who have recurring attacks of severe hypoglycemic symptoms seems of considerable diagnostic value during symptom-free intervals. There is some reason to believe that in such cases the susceptibility to hypoglycemia is familial.—M. B. G.

The effect of insulin on oxidations in isolated muscle tissue. Krebs, H. A., and L. V. Eggleston, Biochem. J. **32**:913. 1938.

The rate of oxygen consumption of minced pigeon breast muscle suspended in phosphate buffer solutions tends to decrease after 30 to 60 minutes, but can be maintained for some time by addition of insulin, especially in presence of citrate and muscle co-enzymes. Traces of zinc inhibit respiration, and zinc insulinate has no favorable effect on respiration; it is concluded that zinc insulinate is not the physiologically-active form of the hormone.—D. L. T.

Action of insulin in cell-free extracts. Lehmann, H., Nature. **141**:690. 1938.

Cell-free muscle extract will form Embden's hexosemonophosphate from glycogen and inorganic phosphate; the rate of this reaction is reduced by addition of minute amounts of insulin, which is thus regarded as an inhibitor of glycogen breakdown.—D. L. T.

Pancreatectomy in the goat. Lukens, F. D. W., Am. J. Physiol. **122**:729. 1938.

Studies made on 4 young completely depancreatized goats showed the fasting-nitrogen excretion slightly increased and the glucose and acetone-body excretion low or absent. In this respect the goat resembles the pig and differs from the dog and cat. The degree of diabetes that develops is about the same as that found in the hypophysectomized-depancreatized dog or cat.—F. A. Hitchcock.

New investigation on the action of insulin. (Neue Untersuchungen zur Frage der Insulin Wirkung). Schur, H., and Elsa Papenheim, Acta med. Scandinav. **95**:167. 1938.

The hourly excretion of phosphorus pentoxide was determined in the urine of 70 subjects before and after giving insulin and sugar. It was concluded that insulin caused a conversion of carbohydrate to fat and a retention of phophate in order to bring about certain phosphorylations in the body. Hypoglycemic reactions do not occur as the direct result of the fall in sugar of the cell fluids but from a destruction of certain cell activities. After hypoglycemic shock has been concluded by sugar, insulin may still remain in an active condition in the organism and bring about further reactions. Large doses of insulin even in the presence of large amounts of sugar can still be dangerous, especially in the form of protamine insulin. Insulin resistance may be due to, *a)*, a strong endogenous sugar formation, *b)*, an insufficiency in fat depots, or *c)*, the antagonistic action of some other substance.—J. M. L.

PARATHYROIDS

Experience with the Hamilton and Highman test for parathyroid hyperfunction in chronic nephritis, toxic goitre, and Paget's disease of bone. Gilligan, D. R., M. C. Volk and S. L. Gargill, J. Clin. Investigation. **17**:641. 1938.

Following the exact technic of the original authors, this group found no evidence of para-

thyroid hyperfunction in 13 of 14 nephritics, in 4 of which postmortem examination showed enlargement and 'secondary' hyperplasia. Positive tests were found in some cases of thyrotoxicosis and Paget's disease of bone. The authors point out that Hamilton and Highman report higher calcium values than most others find in nephritis and phosphorus retention. The reason for failure to confirm the value of the test is unexplained, and the significance of the test is left in question.—E. L. S.

Calcium and phosphorus metabolism in diseases of the thyroparathyroid apparatus. II. Calcium and phosphorus balance (A) following therapeutic radiation of the hyperplastic thyroid gland, and (B) in hyperthyroidic patients treated with iodine. Hansman, F. S., and W. A. Carr Fraser, J. Clin. Investigation **17**:543. 1938.

The diets were high enough in calories to maintain weight equilibrium, high in protein, usually giving positive nitrogen balance, and moderate in supply of calcium and phosphorus. Four-day periods were used for analysis of urine and stool, after a preliminary period on the same diet. Results from 5 untreated cases, 6 under iodine treatment, 12 after the effects of radiation were beginning to be evident, and 4 studied before and after radiation are studied in several ways. There is no correlation established between B.M.R. and the presence of a positive or negative calcium balance. The tendency is usually to a negative balance in untreated cases, which tends to become a positive balance after the effects of irradiation appear, even though the basal rate is still well above normal. The authors think this is due to diminished parathyroid function as a consequence of irradiation.—E. L. S.

The relation of food intake to the development of parathyroid tetany in the rat. Jones, J. H., Am. J. Physiol. **122**:722. 1938.

A total of 108 young and adult parathyroidectomized rats on different diets were studied. A few of the animals which were not very healthy previous to operation refused food, developed tetany, and died within 24 hours. A group (52) of the remaining animals fed a stock diet with a Ca:P ratio of 1.7 was practically all protected from tetany. If after 2 or 3 weeks on this high calcium diet the animals were deprived of food most of them developed tetany within 25 hours, at which time blood analyses showed low calcium and high phosphorus; the feeding of sucrose as the sole dietary constituent afforded partial protection compared to fasting. The majority of rats placed on a fasting diet immediately following parathyroidectomy developed tetany within 24 hours, and showed low Ca and high P in the blood. Sucrose gave a certain degree of protection to such rats, but not as much as a more palatable diet containing protein but only traces of Ca and P. When the conditions tending to produce tetany were brought on slowly by a gradual decrease in the food allowance tetany was still not prevented. The authors therefore believe that food intake is an important factor in the development of parathyroid tetany in the rat, and they also disagree with theory that the simple loss of parathyroid function gives rise to a compensatory mechanism tending to prevent the development of tetany.—S. L. Cohen.

THYROID

On the existence of infundibular nerve centers regulating the function of the thyroid gland (Sur l'existence des centres nerveux infundibulaires réglant la fonction du corps thyroïde). Cahane, M., and Tatiana Cahane, Acta med. Scandinav. **94**:320. 1938.

Rabbits were exposed to cold for 24 to 36 hours and then killed. A decrease in weight of 85 gm. was found. The mean thyroid weight of the treated animals was 10.86 mg./100 gm. of body weight, whereas that of the control animals was 7.90 mg./100 gm. of body weight. Mean weight of the pituitary glands of the two groups per 100 gm. of body weight was 1.25 mg. and 0.90 mg. respectively. An increase in the eosinophile cells was found in the experimental animals. The urine from the treated animals when injected into guinea pigs caused an increase in the weight of thyroid glands: mean per 100 gm. of body weight 19.19 mg., as against 14.05 for the control animals. Similarly, the pituitary weights were 3.76 mg. and 3.30 mg. Exposure to high temperature likewise produced an increase in thyroid weight in the experimental animals. The authors interpret these findings as indicative of two centers in the infundibulum controlling thyroid function through the pituitary, one resulting in stimulation and the other in suppression of thyroid activity. They feel that heat and cold act through the cerebral cortex, and give the name cortico-diencephalo-pituito-thyroid to the reflex.—J. M. L.

Indications for the use of iodine in the treatment of hyperthyroidism. Crile, G. Jr., Med. Clin. N. America **22**:489. 1938.

Iodine is of value in the treatment of hyperthyroidism under the following conditions:

a), In patients with diffuse or nodular goiters in preparation for operation; *b)*, In patients with mild recurrent hyperthyroidism associated with small goiters; *c)*, After operation until the peak of the thyroid reaction is over; *d)*, In small doses for a period of months after operation in patients with extremely hyperplastic glands; *e)*, Between the stages of a divided operation, and *f)*, As a 'therapeutic test' to aid in diagnosis.—I. B.

Size and shape of the heart in hyperthyroidism. A teleroentgenographic study of 200 cases. Gotta, H., Arch. Int. Med. **61**:860. 1938.

The author concludes that hyperthyroidism per se does not cause enlargement of the cardiac area. When a patient with hyperthyroidism has an enlarged cardiac area, it must be assumed that this is due to some complicating cardiovascular disease. Hyperthyroidism can cause enlargement of the cardiac area when there is associated cardiovascular disease. Recovery from hyperthyroidism in such cases may be accompanied by a decrease in the size of the heart and, apart from these cases, recovery from hyperthyroidism is not accompanied by changes in the cardiac area. The bulging of the left middle arch frequently encountered in patients with hyperthyroidism is of constitutional origin and is not a result of hyperthyroidism.—I. B.

Vitamin A and the thyroid. Logaras, G., and J. C. Drummond, Biochem. J. **32**:964. 1938.

Rats receiving 3000 U of vitamin A daily and treated with thyroxin (19 subcutaneous doses of 0.1 mg. in 28 days) showed less loss of weight and much less rise in basal metabolism (25%) than similary-treated rats on a diet free from vitamin A (67% rise in metabolism); the effect of dinitrophenol was independent of the intake of vitamin A. The amount of vitamin A stored in the livers, measured spectrographically, was somewhat greater in animals treated with thyroxin or dinitrophenol than in controls on the same high vitamin intake; the livers of human cases of hyperthyroidism are often rich in vitamin A.—D. L. T.

Hypothyroidism—an etiologic factor in eclampsia. Patterson, W. B., R. E. Nicodemus, and H. F. Hunt, Pennsylvania M. J. **41**:983. 1938.

The authors stress the fact that in pregnancy the thyroid gland is apt to fail in its effort at adjustment to the physiological demands for additional hormone. The resulting hypothyroidism is the cause of goiter and of the toxemia of pregnancy. The authors believe that the high fetal blood cholesterol accompanying fetal hypothyroidism, which is secondary to maternal hypothyroidism, produces placental arterial disease predisposing to thrombosis, placental degeneration, and placental infarction, and that toxins absorbed from the degenerative placental tissue produce the late toxemias of pregnancy. Prophylaxis consists of the administration of iodine and thyroid extract under proper clinical control. Prophylactic is more successful than curative treatment.—I. B.

Exophthalmic goiter. Relation between the blood iodin level and the duration of symptoms in 305 cases. Perkins, H. J., and F. H. Lahey, Arch. Int. Med. **61**:875. 1938.

In this study the iodine level of the blood was correlated with the duration of the symptoms in 305 cases of primary hyperthyroidism (exophthalmic goiter) in which treatment had not been given. It was found that the iodine level of the blood is elevated in the majority of cases of hyperthyroidism in which symptoms have been present from 1 to 9 months. The iodine level tends to fall within the normal range when the syndrome of clinical hyperthyroidism has been present for 1 year or longer.—I. B.

Determination of iodine in sheep thyroid. Sigurjonsson, J., Biochem. J. **32**:945. 1938.

The thyroids of lambs and sheep from Iceland contained approximately 4 mg. iodine per gm. of dried gland.—D. L. T.

Hyperparathyroidism: With special reference to parathyroid adenoma. Washburn, W. W., California and West. Med. **48**:240. 1938.

A case of hyperparathyroidism due to multiple parathyroid adenomas is reported. In patients presenting renal calculi or clinical and x-ray findings of bony pathology simulating osteitis fibrosis cystica, a thorough investigation must be made with the possibility in mind of hyperparathyroidism and parathyroid adenoma. Treatment is essentially surgical.—I. B.

INDEX TO VOLUME 23

ORIGINAL ARTICLES

Acne vulgaris, treatment of with testosterone propionate, 803
Adenomata, pituitary, 200
Adiposity and hypothalamus, 175
Adiposogenital dystrophy, juvenile, 637
Adrenal, cortex, hypertrophy of, 237
  therapy in psychoses, 507
  insufficiency, oral therapy in, 403
  regeneration and carbon tetrachloride, 424
  and thyroid, 419
Adrenalectomy and diuresis, 216
Adrenalin, unusual results following subcutaneous injection of, 366
Adrenals, and blood potassium, 330
  and castration, 524
  effect of androgens on, 339
  and response to cold, 223
  and thyroids and high environmental temperatures, 559
Adreno-genital syndrome, 625
ALTMANN, M., and F. B. HUTT. Estrogens in egg yolk and avian blood calcium, 793
ANDERSON, E., W. HAYMAKER and M. JOSEPH. Hyperadrenocortical syndrome, 398
Androgen, effect of, on female castrates, 243
  and male precocity, 53
  treatment of acne vulgaris with, 803
Androgenic substance, induction of premature puberty with, 661
Androsterone in urine, determination of, 711
Androgens, and comb growth, 44
  effect of, on adrenals, 339
  and the endometrium, 458
  excretion and fate of, 463
  and genital tract of immature female rat, 779
  and lactation, 476
  and metabolism in eunuchoidism, 135
  and sex characteristics, 121
  and uterus, 784
APL hormone and spermatogenesis, 75
Association notice, 666
ASTWOOD, E. B. Quantitative determination of estrogen, 25
Atrophy, fatty, and insulin, 233
Atropine and ovulation, 241
Avitaminoses and the weights of endocrine glands, 575

BALL, J. Estrin and estrus behavior, 197
BARELARE, B. JR. See Richter, C. P., 15
Basal metabolism and estrone, 102
Basophil cells, hyaline change in, not associated with basophilism, 609
BERGMAN, A. J., and C. W. TURNER. Anterior pituitary principles, 228
BERGMAN, H. C. See Glass, S. J., 625
BILLINGS, V. N. See Noble, G. K., 353
BIRNBERG, C. H., L. KURZROK and S. LIVINGSTON. Testosterone propionate and female castrate, 243
BISCHOFF, F., and M. L. LONG. Endocrine factors influencing tumor development, 327
Bitterling, care and handling of, 104
Blood calcium, avian, and estrogens, 793
  potassium and adrenals, 330
  serum calcium level and estrogens, 485
BLOTNER, H. Fatty atrophy and insulin, 233
BOLING, J. L. See also Collins, V. J., 188
BOLING, J. L., W. C. YOUNG and E. W. DEMPSEY. Estrogen, progesterone and heat, 182
Bone, composition and growth of, and hyperthyroidism, 546
BRENEMAN, W. R. Testosterone-propionate and dihydroandrosterone-benzoate and comb growth, 44
BRIEF, B. J. See Sutton, T. S., 211
BRUES, A. M. See Franseen, C. C., 292
BRYAN, A. H. See Kenyon, A. T., 135
BUNDE, C. A. The effects of certain gonadal and gonadotropic hormones on the gestation period of the rat, 345
BUNSTER, E., and R. K. MEYER, Estrogen and hypophysis, 496
BURCH, J. C. See Phelps, D., 458
BUTCHER, E. O., and E. C. PERSIKE, JR. Thymus and pituitary, 501

CAMPBELL, J. Assaying potency of anterior pituitary extracts which increase liver fat, 692
Carbon tetrachloride and adrenal regeneration, 424
Castrate, urine of, effect on testis, 171
Castrates, female, effect of androgen on, 243
Castration and adrenals, 524
CHARVÁT, J., E. KODÍČEK and O. SCHUBERT. Hermaphroditism, 91
CHILES, J. A., JR. See Severinghaus, A. E., 285
Cold, response to, and adrenals, 223
COLE, H. H. See Saunders, F. J., 302
COLE, V. V., and B. K. HARNED. Diabetic traits in a strain of rats, 318
COLLINS, V. J., J. L. BOLING, E. W. DEMPSEY and W. C. YOUNG. Experimental induction of heat, 188
COLLIP, J. B. See also O'Donovan, 718, and Neufeld, A. H., 735
COLLIP, J. B., H. SELYE and J. E. WILLIAMSON. Changes in hypophysis and ovaries of rats chronically treated with an anterior pituitary extract, 279
CONWELL, D. V., and C. J. KURTH. Insulin therapy in mental diseases, 767
Corpora lutea after hypophysectomy, 154
Corpus luteum, effect of, on brooding behavior, 353
Cretinism, treatment of, 364
Cushing's syndrome, 398
CUTTING, W. C. See Emerson, K., JR., 439

DEMPSEY, E. W. See Boling, J. L., 182, and Collins, V. J., 188
Diabetes, in a strain of rats, 318
mellitus, adiposity and the hypothalamus, 175
and hyperthyroidism, 681
Diuresis and adrenalectomy, 216
DONAHUE, J. K. Estrogen and sex cycle, 521
DRIPS, D. G., and A. E. OSTERBERG. Frank method for the determination of prolan, 703
Dysmenorrhea, endocrine theories of, 393

ECKER, A. D. Hyaline change in basophil cells of pituitary not associated with basophilism, 609
EINHORN, N. H. See Rowntree, L. G., 584
EISENBERG, H. See Thorn, G. W., 403
ELLISON, E. T. See Phelps, D., 458
EMERSON, K., JR., See also Thorn, G. W., 403
EMERSON, K., JR., and W. C. CUTTING. Urinary thyrotropic hormone, 439
ENDERLE, E. S. See P. M. Lass, 71
Endocrine factors and tumor, 327
Endocrines, weights of and avitaminoses, 575
Endometrium and testosterone, 458
Epinephrine. See Adrenalin
Estrogen, and estrus behavior, 197
and hypophysis, 496
histology of, 200
progesterone and heat, 182
reaction thresholds of, 64
and sex cycle, 521
substitution in ovariectomized rats, 479
Estrogens, assay of, 25
and avian blood calcium, 793
blood serum calcium level and skeletal changes, 485
in pregnancy, 492
and skeletal changes, 485
in treatment of menopause, 32
and tumor development, 327
and uterine bleeding, 1
Estrone and the metabolic rate, 102
Estrus, behavior, and estrogen, 197
estrogen, progesterone and, 182
experimental induction of, 188
Eunuchoidism, 121
metabolism in, 135

FISHER, C. See Ranson, S. W., 175
FLANDERS, S. See Melchionna, R. H., 468
FLAUM, G. Insulin-insensitivity and the pituitary, 630
FLUHMANN, C. F. Endocrine theories of dysmenorrhea, 393
FOSTER, D. P., and W. L. LOWRIE. Diabetes mellitus and hyperthyroidism, 681
Frank method for the determination of prolan, 703
FRANSEEN, C. C., A. M. BRUES and R. L. RICHARDS. The effect of hypophysectomy on the restoration of the liver following partial hepatectomy in rats, 292

GARDNER, W. U. See Pfeiffer, C. A., 485
GAUNT, R., H. E. POTTS and E. LOOMIS. Post-adrenalectomy diuresis, 216
GEIST, S. H., U. J. SALMON and J. A. GAINES. Testosterone propionate and functional bleeding, 784
Genital tract of female immature rat and testosterone propionate, 779
Gestation, effect of gonadotropic hormones on, 345
GLASS, S. J., and H. C. BERGMAN. Adrenogenital syndrome, 625
Glycogenolysis of surviving rat liver, 751, 760
Gonadotropic, action of urine, 164
Frank method for the determination of prolan, 703
hormones and corpora lutea after hypophysectomy, 154
Gonadotropins, light and ovarial development, 618
GRAY, H. See G. B. Robson, 800
GREEP, R. O. See also Hisaw, F. L., 1
Corpora lutea after hypophysectomy, 154
Growth and thyroids, 446

HAMILTON, J. B. Male precocity and synthetic male hormone substance, 53
HARNED, B. K. See Cole, V. V., 318
HARRISON, A. See Lawrence, C. H., 360
HAYMAKER, W. See Anderson, E., 398
HELLER, C. G. See Lauson, H., 479
Hermaphroditism, 91
HIGGINS, G. M. See also Ingle, D. J., 419
HIGGINS, G. M., and D. J. INGLE. Carbon tetrachloride and adrenal regeneration, 424
HISAW, F. L., and R. O. GREEP. Inhibition of uterine bleeding with estradiol and progesterone, 1
HORVATH, S. M. Adrenals and response to cold, 223
HUTT, F. B. See Altmann, M., 793
Hyperadrenocortical syndrome, 398
Hyperthyroidism, bone growth, and composition, 546
and diabetes mellitus, 681
Hypophysectomy, effect of, on hypothalamus, 263
corpora lutea after, 154
of the mouse, 99
Hypophysis, anterior lobe extract of, in treatment of cretinism, 364
carbohydrate and fat metabolism hormones, 735
and development of human uterus, 598
effect of A P E on cellular changes in, 279
and estrogen, 496
extract in pituitary dwarfism, 360
extracts which increase liver fat, assay of, 692
gonadotropic hormones of, 302
and gestation period, 345
histology of, and estrin, 200
hyaline change in basophil dells, 609
innervation of, 263
and insulin-insensitivity, 630
lactogenic and carbohydrate metabolism hormones, 228
and liver growth, 292
metabolic principle and the melanophore hormone, 718

in protein metabolism, 368
and thymus, 501
thyroids and growth, 446
thyrotropic hormone assay, 429
use of ultra-centrifuge in separation and purification of hormones of, 285
and vitamin A, 211
weight of, and tumor, 87
Hypothalamus and adiposity, 175
relation of, to hypophysis, 263

Infantilism, congenital webbed neck and cubitus valgus, 566
INGLE, D. J. See also Higgins, G. M., 424
INGLE, D. J., and G. M. HIGGINS. Thyroxine and adrenal regeneration, 419
INGRAM, W. R. See Ransom, S. W., 175
Insulin, and fatty atrophy, 233
oral administration of, 535
insensitivity and the pituitary, 630
therapy in mental diseases, 767
Iodine-reducing substances of thymus extract, 584, 593

JOSEPH, M. See Anderson, E., 398

KENYON, A. T. Androgen and sex characteristics, 121
KENYON, A. T., I. SANDIFORD, A. H. BRYAN, K. KNOWLTON and F. C. KOCH. Metabolism in eunuchoidism, 135
KOCHAKIAN, C. D. Excretion and fate of androgens, 463
KODÍČEK, E. See Charvát, J., 91
KROC, R. L. Adrenals and castration, 524
KUMPF, K. F. See Noble, G. K., 353
KUNSTADTER, R. H. Induction of premature puberty with androgenic substance, 661
KURTH, C. J. See Conwell, D. V., 767
KURTZ, G. See Szarka, A. J., 64
KURZROK, L. See Birnberg, C. H., 243
KURZROK, R. See also Lass, P. M., 39, 71, Wiesbader, H., 32 and Wilson, L., 79
KURZROK, R., and C. P. O'CONNELL. Testosterone and lactation, 476

Lactation, human ovulation during, 39
nutritional requirements in, 15
and testosterone propionate, 476
Lactogenic hormone, 228
LASNITZKI, M. See Twort, J. M., 87
LASS, P. M., E. S. ENDERLE and R. KURZROK. Skin tests for pregnancy, 71
LASS, P. M., J. SMELSER and R. KURZROK. Human ovulation during lactation, 39
LAUSON, H., C. G. HELLER and E. L. SEVRINGHAUS. Estradiol substitution in ovariectomized rats, 479
LAWRENCE, C. H., and A. HARRISON. Pituitary dwarfism, 360
LEATHEM, J. H., and J. A. MORRELL. Gonadotropic action of urine, 164
LEVIN, L. See Severinghaus, A. E., 285
Light, gonadotropins and ovarial development, 618
Liver, effect of hypophysis on growth of, 292
fat, and anterior pituitary extracts, 692
LIVINGSTON, S. See Birnberg, C. H., 243
LOEHNER, C. A. Adrenal cortex therapy in psychoses, 507
LONG, M. L. See Bischoff, F., 327
LOOMIS, E. See Gaunt, R., 216
LOWRIE, W. L. See Foster, D. P., 681

MCCLENDON, J. F., Estrone and the metabolic rate, 102
MACKAY, E. M., and L. L. Hypertrophy of adrenal cortex, 237
MCLEAN, F. C. See Smith, E. E., 546
MAKEPEACE, A. W. Atropine and ovulation, 241
Male precocity and synthetic male hormone substance, 53
MARENZI, A. D. Blood potassium and suprarenal glands, 330
MELCHIONNA, R. H., and S. FLANDERS. Ocular transplant of seminal vesicle, 468
Menopause, symptoms and treatment of, with estrogens, 32
Mental diseases, insulin therapy in, 767
Metabolism, carbohydrate, anterior pituitary hormone, 228
fat and pituitary extracts, 735
in eunuchoidism, 135
rate of, and estrone, 102
MEYER, R. K. See Bunster, E., 496
MITTELMANN, B. Adiposogenital dystrophy, 637
MOFFAT, W. M. Specific dynamic action test in castrated and menopausal women, 747
MOLITCH, M. Treatment of acne vulgaris with testosterone propionate, 803
MORRELL, J. A. See Leathem, J. H., 164
Myometrial cycle, human, 79

Naso-genital relationship, 58
NEUFELD, A. H., and J. B. COLLIP. Pituitary extracts, carbohydrate and fat metabolism, 735
NEUSTADT, R. Photo-colorimetric determination of androsterone in urine, 711
NOBLE, G. K., K. F. KUMPF and V. N. BILLINGS. The induction of brooding behavior in the Jewel Fish, 353

O'CONNELL, C. P. See Kurzrok, R., 476
Oral medication, 535
O'DONOVAN, D. K., and J. B. COLLIP. Metabolic principle of the pituitary and the melanophore hormone, 718
OSGOOD, E. E. Pituitary cachexia, 656
OSTERBERG, A. E. See Drips, D. G., 703
Ovarial development, 618
Ovariectomized rats, estrogen substitution in, 479
Ovary, effect of A P E on weight of, 279
Ovulation and atropine, 241
human, during lactation, 39

PARKER, F., JR., and B. TENNEY, JR. Estrogens in pregnancy, 492
PASCHKIS, K. E. Hypophysis in protein metabolism. Is the pituitary factor active

in protein metabolism identical with the growth hormone?, 368
PAYNE, S. A., and E. K. SHELTON. Anterior pituitary sex fraction and uterine development, 598
PERSIKE, E. C., JR. See Butcher, E. O., 501
PFEIFFER, C. A., and W. U. GARDNER. Skeletal changes and estrogens, 485
PHELPS, D., J. C. BURCH and E. T. ELLISON. Testosterone and guinea-pig endometrium, 458
Pituitary cachexia, 656
dwarfism, treatment in, 360
POTTS, H. E. See Gaunt, R., 216
Pregnancy, estrogens in, 492
nutritional requirements in, 15
skin tests for, 71
Progesterone, estrogen and heat, 182
and uterine bleeding, 1
Prolactin, effect of, on brooding behavior, 353
Protamine insulin, 800
Protein metabolism, hypophysis in, 368
Psychoses, adrenal cortex therapy in, 507
Puberty, premature, induction of with androgenic substance, 661

RANSON, S. W., C. FISHER and W. R. INGRAM. Hypothalamus and adiposity, 175
RASMUSSEN, A. T. Innervation of the hypophysis, 263
RICHARDS, R. L. See Franseen, C. C., 292
RICHTER, C. P., and B. BARELARE, JR. Nutritional requirements of pregnant and lactating rats, 15
RILEY, G. M., and E. WITSCHI. Light, gonadotropins and ovarial development, 618
ROBSON, G. B., and H. GRAY. Protamine insulin, 800
ROSEN, S. See Shelesnyak, M. C., 58
ROTHBURD, C. M. See Weisman, A. I., 104
ROWNTREE, L. G. See also Schaffer, N. K., 593
ROWNTREE, L. G., A. STEINBERG, N. H. EINHORN and N. K. SCHAFFER. Iodine-reducing substances of thymus extract, 584
RUBINSTEIN, H. S. APL hormone and spermatogenesis, 75
Effect of castrate urine on testis, 171
RUDOLF, G. DE M. The treatment of a cretin with anterior pituitary extract, 364
Unusual results following the injection of epinephrin, 366

SALMON, T. N. Growth rate, thyroids, parathyroids and hypophysis, 446
SALMON, U. J. See also Geist, S. H., 784
SALMON, U. J. Testosterone propionate and the genital tract of the immature female rat, 779
SALTER, W. T. See Wilson, H., 535
SANDIFORD, I. See Kenyon, A. T., 135
SAPPINGTON, T. S. See Wilson, H., 535
SAUNDERS, F. J., and H. H. COLE. On the reliability of present methods for characterizing two gonadotropic hormones, follicle-stimulator and luteinizer, 302
SCHAFFER, N. K. See also Rowntree, L. G., 584
SCHAFFER, N. K., W. M. ZIEGLER and L. G. ROWNTREE. Iodine-reducing substances of thymus extract, 593
SCHMIDT, E. C. H., JR. See Starkey, W. F., 339
SCHMIDT, L. H., and I. G. Environmental temperature and the action of thyroxine, 553, 559
SCHUBERT, O. See Charvát, J., 91
SECKEL, H. P. G. Glycogenolysis of surviving rat liver, 751, 760
SELYE, H. See Collip, J. B., 279
Seminal vesicle, ocular transplant of, 468
SEVERINGHAUS, A. E., L. LEVIN and J. A. CHILES, JR. Hormone studies with the ultra-centrifuge, 285
SEVRINGHAUS, E. L. See Lauson, H., 479
Sex cycle and estrogen, 521
SHELESNYAK, M. C., and S. ROSEN. Nasogenital relationship, 58
SHELTON, E. K. See Payne, S. A., 598
SMELSER, G. K. Thyrotropic hormone assay, 429
SMELSER, J. See Lass, P. M., 39
SMITH, E. E., and F. C. MCLEAN. Hyperthyroidism and bone, 546
Specific dynamic action test in castrated and menopausal women, 747
Spermatogenesis and APL hormone, 75
STARKEY, W. F., and E. C. H. SCHMIDT, JR. The effect of testosterone-propionate on the x-zone of the mouse adrenal, 339
STEINBERG, A. See also Rowntree, L. G., 584
Thymus extract, 581
SURE, B. Avitaminoses and weights of endocrine glands, 575
SUTTON, T. S., and B. J. BRIEF. Hypophysis and vitamin A, 211
SZARKA, A. J., and G. KURTZ. Reaction thresholds, of estrogen, 64

Temperature, environmental, and the action of thyroxine, 553, 559
TENNEY, B., JR. See Parker, F., Jr., 492
Testis, effect of castrate urine on, 171
extract in pituitary dwarfism, 360
THOMAS, F. Hypophysectomy of the mouse, 99
THORN, G. W., K. EMERSON, JR., and H. EISENBERG. Oral therapy in adrenal insufficiency, 403
Thyroid and adrenal regeneration, 419
extract in treatment of cretinism, 364
hyperthyroidism and diabetes mellitus, 681
protein and insulin, oral administration, 535
Thyroids, adrenals and high environmental temperatures, 559
and growth, 446
Thyrotropic hormone, assay of, 429
from urine, 439
Thyroxine, action of and environmental temperature, 553, 559

Thymus, extract, 581, 584, 593
and pituitary, 501
TURNER, C. W. See Bergman, A. J., 228
TURNER, H. H. A syndrome of infantilism, congenital webbed neck and cubitus valgus, 566
TWORT, J. M., and M. LASNITZKI. Tumor and pituitary weight, 87

Urine, androsterone in, determination of, 711
of castrate, effect on testis, 171
gonadotropic action of, 164
thyrotropic hormone from, 439
Uterine bleeding, inhibition of, by estrogens, 1
Uterus, development of and anterior pituitary sex fraction, 598
and testosterone propionate, 784

Vitamin A and hypophysis, 211

WEISMAN, A. I., and C. M. ROTHBURD. Care and handling of the bitterling, 104
WIESBADER, H., and R. KURZROK. The menopause, 32
WILLIAMSON, J. E. See Collip, J. B., 279
WILSON, H., T. S. SAPPINGTON and W. T. SALTER. Oral administration of hormonal proteins, thyroid and insulin, 535
WILSON, L., and R. KURZROK. Human myometrial cycle, 79
WITSCHI, E. See Riley, G. M., 618
WOLFE, J. M., and A. W. WRIGHT. Estrin and pituitary histology, 200

YOUNG, W. C. See Boling, J. L., 182, and Collins, V. J., 188

ZIEGLER, W. M. See Schaffer, N. K., 593

ABSTRACTS

ABE, K. See Satake, Y., 381
Abortion, sex hormone factors in, 534
treatment of with progesterone, 254
ABRAMOWITZ, A. A., and R. K. Chromatophoric hormone, 251
Acne vulgaris, endocrine therapy in, 668
Acromegaly, heart in, 805
ADAMS, A. E., and G. TUKEY. Effect of administering frog anterior pituitary substance to immature female mice, 384
ADAMS, F. M. See Wade, H. K., 258
ADDIS, T., D. KARNOFSKY, W. LEW and L. J. POO. Protein content of the body after administration of thyroxine and dinitrophenol after thyroidectomy, 390
Addison's disease, 109
desoxycorticosterone acetate in, 676
Adiposity, girdle type and mentally deficient males, 677
Adrenal, cortex, chemical studies of, 381
methods for identification of compounds of, 381
treatment of marasmus with, 675
cortical extract, in cardiac insufficiency, 382
and oxygen consumption, 108
and renal function, 110
in rest and work, 111
cortical hormone and fertility, pregnancy and lactation, 251
deficiency, 250, 676
denervation, paralytic secretion from medulla after, 380
secretion, and eserin, 109
and potassium, 109
transplants, 380
tumor, 380
and steroids in urine, 380
virilism and intersexuality, 379
Adrenalectomized toads, survival of, 675
Adrenalectomy, action of nicotine and blood pressure, 676
and blood phospholipids and total fatty acids, 111
and ketolytic activity, 251
and renal function, 110
and water exchange, 251
Adrenalin, adrenals and nicotine, 676
effect, on blood sugar, 111
of cold upon secretion of, 381
of, on heart rate, 380
of sex hormones on vascular effects of, 381
of sodium nitrite on, 381
estimation of, 676
and hyperglycemia in emotional excitement, 111
and lack of response to insulin in diphtheria intoxication, 674
load, ammonium chloride and magnesium sulphate, 531
lung edema, 530
and muscular work, 531
occurrence and action of in ovaries, 114
and plasma potassium level, 110
and potassium, action of, on arterial blood pressure, 109
replacement of, and splanchnic nerves, 530
and toad's heart, 530
ADRENALS, absence of and B_2, B_4, and B_6, 109
calcium and potassium of blood after excision and grafting of, 675
cortin and antagonistic factor in serum, 676
and liver fat, 806
and work-performance, 806
effect of castration and androgens on, 116
of pregnancy and lactation on weight of, 381
of Na and K on weight of, 381
epinephrine and nicotine, 676
homeoplastic transplantation of, in rats of inbred strains, 110
and hyperglycemic action of lobelin, 531
hypertrophy and hypoplasia of, 250
sensitization of, 251
splanchnic nerves and work capacity, 531
Adrenin, effect of, on arteries, 533
AINSWORTH, M. L., S. D. EDELMAN and R. I. FRIED. Thrombocytopenic purpura hemorrhagica, parathyroid hormone and calcium gluconate in, 389
ALDER-MÖNNICH, J., and R. TIBERI. Thyroid and female sex organs, 390
Allen-Doisy test, 678
ALLOTT, E. N., and J. JEMSON. Generalized osteitis-fibrosa and parathyroid tumor, 389
Amenorrheas, hormone treatment of, 531
Amino acids, calorigenic action of, in hypophysectomy, 259
ANDERSEN, D. H. See also Victor, J., 252, 392
ANDERSEN, D. H., and J. VICTOR. Amniotin or theelin and metabolism of liver and kidney, 252
ANDERSON, C. M. The anterior pituitary gland and carbohydrate metabolism, 384
Androgen, birth and prenatal development, 533
and descent of testes, 532
and the effect of adrenin on arteries, 533
Androgenic, hormone, and muscular hypertrophy, 256
factors influencing effectiveness of, 679
sterols, hypophysectomy and castration, 115
Androgens, activation of testosterone by higher fatty acids, 114
and blood lipids, 116
castration and the adrenals, 116
and glands of prepuce, 253
rapid test for, 533
and seminal vesicles, 256
synthetic male hormone re prostatic hypertrophy, 679
in treatment of mastopathies, menstrual and menopausal disturbances, 532
in urine, estimation of, 680
ANERSEN, I., and K. PEDERSEN-BJERGAARD. Parallel determination of urinary estrogens, 678
Antianemic function of small intestine, 678
Antigonadotropic sera, 809
ARAYA, R. Thyroid and metropathia hemorrhagica, 252
Arrhenoblastoma, 110
ASHWORTH, U. S., and G. R. COWGILL. Endogenous nitrogen and metabolism relationships in hypophysectomized rats, 805
Assay, biological standardization, 245
Autonomic, drugs, influence on ejaculation, 533
nervous system and norepinephrine, 111

BARKS, O. L., and M. D. OVERHOLSER. Hyperplasia and hypertrophy of the uterine musculature in ovariectomized rats, 382
BARNES, R. H. See MACKAY, 251
Basedow's disease, muscle changes in, 391
Basophilism, surgical treatment of, 385
BAXTER, J. H., JR. See Greer, C. M., 111
BEAMER, C., and G. S. EADIE. Epinephrine and lack of response to insulin in diphtheria intoxication, 674
BEARD, H. H., and P. PIZZOLATO. Purines and creatine-creatinine metabolism, 676
BEILLY, J. S. See Rascoff, H., 388
BELONOSCHKIN, B. Hormonal treatment of amenorrheas, 531
BENAGLIA, A. E. See Bodo, R. C., 111
BERGMAN, A. J., and C. W. TURNER. The biological assay of the carbohydrate metabolism hormone of the anterior pituitary, 384
BEST, C. H. See also McLean, W. L., 807
BEST, C. H., and J. H. RIDOUT. Pancreas and liver fat, 262
Biological standardization, 245
BISCHOFF, F. Gonadotropic extracts and zinc and copper extracts, 115
BISHOP, P. M. F. Clinical experiment in estrin therapy, 679
BJERING, B. Investigation of the diabetogenous hormone in urine, 382
BLANCHARD, E. W. See Yeakel, E. H., 111
BLANTON, J. L. See Crile, G., Jr., 390
BLATHERWICK, N. R., M. E. EWING and P. J. BRADSHAW. Zinc and iron salts and the hypoglycemic action of insulin, 117
BLATT, H. See Cope, O., 259
BLEYER, A. Advanced paternal age in causing Mongolism, 676
Blood, calcium and potassium after excision and grafting of adrenals, 675
lipids, and androgens, 116

and anterior pituitary lobe extract, 113
and insulin treatment of schizophrenia, 262, 388
maternal and fetal circulation, estrogens in, 117
phospholipids, total fatty acids, in adrenalectomy, 111
pressure, nicotine and adrenalectomy, 676
thyroxine and adrenals, 391
red cell count and menstrual cycle, 116
serum sodium content in myxedema, 120
sugar, curve after glucose or insulin and cut vagi, 387
effect of sympathin on, 111
raising principle in urine, 382
and sympathin in emotional excitement, 111
uric acid in, and insulin, 388
BODO, R. C., and A. E. BENAGLIA. Effect of sympathin on blood sugar, 111
BOGDANOVITCH, S. B., and E. B. MAN. Sex hormones and lipoids, 252
Bone, variations in, of growing rats and parathyroid extract, 389
BORGSTRÖM, S. Magnesium content of serum and prolan, 252
BOSSELMANN, H. Intersexuality and suprarenal virilism, 379
BOYD, E. M., and G. M. BROWN. Water uptake of frogs and posterior hypophysis, 250
BRADSHAW, P. J. See Blatherwick, N. R., 117
BRANNON, E. S. See Greer, C. M., 111
BREA, A. M., and C. C. S. GANDARA. Lactation and follicular hormone, 115
BRIDGE, E. M. Insulin and glycogen reserves, 262
BRITTON, S. W. See also Silvette, H., 110
BRITTON, S. W., H. SILVETTE and R. KLINE. Adrenal insufficiency, 250
BROOKS, C. M. Pituitary ovulation, 252
BROWN, G. M. See Boyd, E. M., 250
BRUCH, H. See McCune, D. J., 678
BRUNO, A. A. See Cuatrecasas, J., 386
BUILLARD, H., and A. RAVINA. Male hormone and glands of prepuce, 253
BURCH, J. C. See Diaz, J. T., 109, 254
BURN, J. H. Biological standardization, 245
BURROWS, R. B. Variations produced in bones of growing rats by parathyroid extracts, 389
BUTLER, G. C., and G. F. MARRIAN. Chemical studies on the adreno-genital syndrome, steroids in urine, 380

CAFFIER, P. Estrogens and fallopian tube mucosa, 253
CAHANE, M., and T. Infundibular nerve centers and the thyroid, 811
CAMERON, D. E. See Randall, L. O., 262
CAMPBELL, E. H. Status lymphaticus, 675
CAPITAIN. See Desmarest, 532
CARTLAND, G. F., and F. W. NELSON. Bioassay of mare serum hormone, 253
CARY, F. S. Synthetic male hormone re prostatic hypertrophy, 679
Castration and androgenic sterols, 115
CATTANEO, M. Pituitary thyreostimulin, 390
CAUSSADE, M. G. Hypertrophy and hyperplasia of the adrenals, 250
CHAMBERLIN, P. E. See Hall, V. E., 251
CHAMPY, C. Ovarian tumors and folliculin, 253
CHARNY, C. W. See Mazer, C., 677
CHEN, G., and H. B. VAN DYKE. Merthiolate and gonadotropic effects, 258
Children, adolescent, handbook of methods for the study of, 245
public school, data on the growth of, 245
Chorionepithelioma, hormonal findings, 531
CHOWN, B., and M. LEE. Renal rickets, dwarfism and pituitary, 259
CHRISTENSEN, J. T. See Jacobsen, E., 256
Chromatophorotropic hormone, 251
CLAUBERG, C. Artificially stimulated tubal growth, 254
CLAUBERG, C., and Z. UBESTÜN. Orally induced menstruation and synthetic sex hormones, 679
Climacteric and oophorectomy, 257
COLOMBO, E. See Peralta, R. A., 257
COON, J. M. A new method for posterior pituitary assay, 384
COOPERSTOCK, M. Laurence-Moon-Biedl syndrome, 677
COPE, C. L. The effect of antithyrotropic serum on the action of human thyrotropic hormone, 384
COPE, O., A. HAGSTRÖMER and H. BLATT. Blood serum amylase and hypophysectomized dog, 259
COREY, E. L. See Ehrenstein, M., 114
Corpus luteum, graafian follicle and gonadotropins, 255
Corticosterone, chemical structure of, 676
Cortin, assay of, 250
and liver fat, 806
and work-performance, 806
CORYN, G. Endocrine affections of the skeleton, the thyroid, 390
COSGROVE, S. A. Hormonal findings in hydatidiform mole and chorionepitheliomas, 531
COURVILLE, C., and V. R. MASON. The heart in acromegaly, 805
COWGILL, G. R. See Ashworth, U. S., 805
Creatine tolerance test in diagnosis of Grave's disease, 392
Cretinoid epiphyseal dysgenesis, 392
CRILE, G., JR. Iodine in the treatment of hyperthyroidism, 811
CRILE, G., JR., and J. L. BLANTON. Exophthalmic goiter, 390
Cryptorchidism and hypogonadism, endocrine study of, 680
treatment of, 117, 383
treatment of by pituitary hormones, 119
CUATRECASAS, J., and A. A. BRUNO. Biochemical mechanism of insulin and metrazol shock, 386
Cuboni reaction, 678
CURRIER, R., and G. GROS. Ovarian hormones, 254
Cushing's syndrome, surgical treatment of, 385
CUTULY, E., D. R. MCCULLAGH and E. CUTULY. Androgenic sterols, hypophysectomy and castration, 115

D'AMOUR, F. E., and D. FUNK. Assay of cortin, 250
D'AMOUR, M. C., and F. E. Assay of gonad-stimulating preparations, 254
DANDY, W. E., and F. L. REICHERT. Experimental hypophysectomy of dogs, 259
DANIELS, L. P. Exophthalmos in Grave's disease, 391
DAVIS, H. P. See Reece, R. P., 808
DAVIS, M. E., and A. K. KOFF. Experimental production of ovulation, 679
DAY, H. G. See Greulich, W. W., 245
DEANESLY, R., and A. S. PARKES. Factors influencing the effectiveness of administered hormones, 679
DEARBORN, W. F., J. W. M. ROTHNEY and F. K. SHUTTLEWORTH. Data on the growth of public school children, 245
DEL CASTILLO, B. E., and R. SAMMARTINO. Prolonged treatment with estrone, 113
Dermatophytosis in diabetes, 387
DESCLIN, L., and CH. GRÉGOIRE. Lactation and gonadotropic function of hypophysis, 805
DESMAREST and CAPITAIN. Mastopathies, menstrual and menopausal disturbances treated with testosterone, 532
Diabetes, insipidus, 251
dystocia in, 806
urea clearance of cats with, 259
voluntary ingestion of water in normals and in individuals with, 385
insulin and protamine zinc insulin, 388
juvenile, protamine insulin in the treatment of, 118
mellitus, and congenital aplasia of islands of Langerhans, 262
end results in uncontrolled, 386
pancreatic and thyroidectomy, 809
phloridzin, and pancreatic function, 118
significance of dermatophytosis in, 387
syphilitic, 387
Diabetic, children, mortality of, 118
exophthalmos dystosis and xanthomatosis, 389
heart and insulin hypoglycemia, 118
DIAZ, J. T., D. PHELPS, E. T. ELLISON and J. C. BURCH. Effects of gonadotropic substances and estrin, 109
Gonadotropins and estrin, effects on ovaries, pituitaries and adrenals, 254
DILL, D. B. See Missiuro, V., 111

DILLON, E. S., and W. W. DYER. End results in uncontrolled mild diabetes, 386
DINGEMANSE, E., and E. LAQUEUR. Estimation of comb growth hormone in urine, 680
Estrone in human male urine, 680
Diphtheria intoxication, epinephrine and lack of response to insulin in, 674
DOHAN, F. C., and F. D. W. LUKENS. Thyroidectomy and pancreatic diabetes
DONATO, D. Calcium and potassium in blood after excision and grafting of adrenals, 675
DRUMMOND, J. C. See Logaras, G., 812
DUNN, R. D., and F. J. NORTHWAY. Visscher-Bowman pregnancy test, 115
DU VIGNEAUD, V. See also Irving, G. W., 806
DU VIGNEAUD, V., G. W. IRVING, JR., H. M. DYER and R. R. SEALOCK. Electrophoresis of posterior pituitary preparations, 112
Dwarfism and renal rickets, 259
DYER, W. W. See Dillon, E. S., 386
DYKE, C. G. See Wortis, S. B., 389

EADIE, G. S. See Beamer, C., 674
Eclampsia, effect of blood of, on urinary output and blood pressure, 384
and hypothyroidism, 812
EDELMAN, S. D. See Ainsworth, M. L., 389
EDWARDS, H. T. See Missiuro, V., 111
EGGERT, B. The ultimobranchial body, 805
EGGLESTON, L. V. See Krebs, H. A., 810
EHRENSTEIN, M., and E. L. COREY. Activation of testosterone by higher fatty acids, 114
Ejaculation, influence of autonomic drugs on, 533
ELDEN, C. A. Treatment of abortion with progesterone, 254
ELEFTHERIOU, D. S. Transplantation of the uterus, 532
ELLISON, E. T. See Diaz, J. T., 109, 254
Emaciation, pubertal, 807
Endoangiitis obliterans and ulcers, treatment with sex hormones, 258
Endocrine therapy in acne vulgaris, 678
ENGEL, P. Irradiation and substances resembling follicular hormone, 532
Enterocrinin, 112
Epinephrine. See adrenalin
ERSPAMER, V. Chromaffine and argentaffine cells in pancreas, 262
ESCAMILLA, R. F., and H. LISSER. Simmonds's disease, 805
Eserin and adrenal secretion, 109
Estrogen, in local treatment of pruritus vulvae, 534
in male urine, 680
therapy, 679
treatment of vaginitis with, 258
Estrogenic diols from urine of pregnant mares, 116
hormone, effect of on contractility of fallopian tubes, 680
hormones, factors influencing effectiveness of, 679
substances, 532
and sarcomas, 533
Estrogens, effect of, on metabolism of liver and kidney, 252
prolonged treatment with, 113
on uterine musculature, 382
estrin and the effects of gonadotropic substances, 109
and fallopian tube mucosa, 253
and fat metabolism, 258
and hypophyseal hormones, 807
and the immature alligator, 532
and lactation, 114, 115
in maternal and fetal circulation, 117
in peripheral gangrene, 533
in therapeutic baths, 117
urinary, determination of, 678
use of, in initiating labor, 383
ETCHEVERRY, A. O. Influence of cutting the vagi on the blood sugar curve after glucose or insulin, 387
EULER, U. S. v., and S. HAMMARSTRÖM. Occurrence and action of adrenalin in ovaries, 114
EVANS, H. M., J. M. LUCK, R. I. PENCHARZ and H. C. STONER. Amino acids in the hypophysectomized animal, 259
EWING, M. E. See Blatherwick, N. R., 117
Exophthalmic goiter, 390
and blood iodine level, 812
pharyngeal infections in, 391
Exophthalmos in Grave's disease, 391

FALK, E. A. See Papanicolaou, G. N., 256
Fallopian tube, and estrin, 254
contractility of, and estrogenic hormone, 680
mucosa and estrogens, 253
FALLS, F. H. Progestin in obstetrical complications, 255
FALTA, W. Protamine zinc insulin and insulin resistance, 387
FARR, L. E., K. HARE and R. A. PHILLIPS. Urea clearance and diabetes insipidus, 259
FASCIOLO, J. C. See Houssay, B. A., 111
FELLOWS, M. D. See Messinger, W. J., 117
Fertility and adrenal cortical hormone, 251
and menstrual cycle, 114
FEVOLD, H. L. See Foster, M. A., 255
FINK, H. See Friedman, J. J., 806
FISHER, C., H. W. Magoun and S. W. Ranson. Dystocia in diabetes insysidus, 806
FOGLIA, V. G. See Houssay, B. A., 118
FOLLEY, S. J., and S. K. KON. Effect of sex hormones on lactation, 680
Follicle hormone, histologic demonstration of activity of, 258
Follicular development, ovulation and gonadotropins, 255
hormone and tubal growth, 254
Folliculin, hypophysis and thyroid, 259
and ovarian tumors, 253
and pregnancy, 257
Folliculophasic hormones, 680
FORBES, T. R. Estrone and the immature alligator, 532
FOSTER, M. A. Gonadotropins, graafian follicle and corpus luteum, 255
FOSTER, M. A., and H. L. FEVOLD. Gonadotropins, follicular development and ovulation, 255
FRADA, G. Pregnancy and glucemic response to gonadotropins, 260
FRANCK, S. Hypophysis, thyroid and folliculin, 259, 260
FRANK, C. W., and P. B. WAHRSINGER. Skin tests for pregnancy, 116
FRASER, W. A. C. See Hansman, F. S., 811
FREUD, J. Ovotrophic and folliculophasic hormones, 680
FRIED, R. I. See Ainsworth, M. L., 389
FRIEDMAN, J. J., and H. FINK. Intradermal test for pregnancy, 806
FUCHS, R. T. An initial depression of heart rate in response to epinephrine in human subjects, 380
FUNK, D. See D'Amour, F. E., 250
FUSTINONI, O. Survival of adrenalectomized toads, 675

GALLO, F. N. See Lewis, J. T., 109
GALTSOFF, P. S. Physiology of reproduction of Ostrea virginica, 532
GÁNDARA, C. C. S. See Brea, A. M., 115
Gangrene, estrogens in prevention of, 533
GARGILL, S. L. See Gilligan, D. R., 810
GEIST, S. H., U. J. SALMON and M. MINTZ. Effect of estrogenic hormone on the contractility of the fallopian tubes, 680
Genitals, male, affections of, and gonadotropic hormone therapy, 117
GERLEI, F. Thyroxine, adrenals and blood pressure, 391
GERSCH, I. Antuitrin-S cutaneous test for pregnancy, 116
GILLIGAN, D. R., M. C. VOLK and S. L. GARGILL. Test for hyperfunction of parathyroid, 810
Goiter, exophthalmic, 390
and pharyngeal infections, 391
GOLDBERG, I. Plasma proteins in pituitary insufficiency, 806
Gonadotropic, effects of hypophysis, 258
extracts and zinc and copper salts, 115
hormone, of pregnancy urine in male sexual underdevelopment, 115
therapy in cryptorchidism and disturbances of spermatogenesis, 117
Gonadotropins, assay of, 256
effects of, and estrin, 109
and estrin, effects on ovaries, pituitaries and adrenals, 254
follicular development and ovulation, 255

glucemic response to, and pregnancy, 260
graafian follicle and corpus luteum, 255
Gonad-stimulating preparations, assay of, 254
Gonads and tissue metabolism, 392
GORDON, M. B. Endocrine obesity in children, 677
endocrine study of hypogonadism and cryptorchidism, 680
GOTTA, H. Heart in hyperthyroidism, 812
Graafian follicle, corpus luteum and gonadotropins, 255
GRAFFAGUINO, P., and E. VON HAAM. Skin tests for pregnancy, 119
GRAY, C. H. Ketogenic activity of endocrine extracts, 806
Grave's disease, 391
creatine tolerance test in diagnosis of, 392
GREER, C. M., J. O. PINKSTON, J. H. BAXTER, JR. and E. S. BRANNON. Nor-epinephrine as a mediator in the sympathetic division, 111
GRÉGOIRE, CH. See Desclin, L., 805
GREULICH, W. W., H. G. DAY, S. E. LACHMAN, J. B. WOLFE and F. K. SHUTTLEWORTH. handbook of methods for the study of adolescent children, 245
GROS, G. See Currier, R., 254
GROSH, L. C. See Malamud, N., 388
Growth of public school children, data on, 245
GRUBBS, R. C. See Hitchcock, F. A., 108
GUTHMANN, H., and H. VETTER. Sterility of women, 114
GUTKELCH, A. N., and S. ZUCKERMAN. Red cell count and menstrual cycle, 116

HAGSTRÖMER, A. See Cope, O., 259
Hall, K., and V. KOVENSCHENSKY. Effects of castration and androgens on adrenals, 116
HALL, V. E., P. E. CHAMBERLIN and O. H. MÜLLER. Adrenal cortical hormone and fertility, pregnancy and lactation, 251
HAMILTON, J. B. Effect of male hormone on descent of testes, 532
HAMILTON, J. B., and J. M. WOLFE. Effect of male hormone substances on birth and prenatal development, 533
HAMMARSTRÖM, S. See Euler, U. S. v., 114
HANSMAN, F. S., and W. A. C. FRASER. Calcium and phosphorus metabolism in diseases of thyroparathyroid apparatus, 811
HARE, K. See Farr, L. E., 259
HARRISON, A. M. See Lawrence, C. H., 115
HARTMAN, A. F., and J. C. JAUDON. Hypoglycemia, 810
HATHAWAY, I. L. See Reece, R. P., 808
Heart in acromegaly, 805
HECKER, A. O., and V. C. WARREN. Girdle type adiposity among mentally deficient males, 677
HELLER, C. G., H. LAUSON and E. L. SEVRINGHAUS. Assay of gonadotropins, 256
HERMANN, H., F. JOURDAN, G. MORIN and J. VIAL. Is there a paralytic secretion from the medulla of a permanently denervated adrenal, 380
HEROLD, L. Inhibiting action of follicle hormone on mammary glands of lactating rats, 114
HIGGINS, G. M. See also Ingle, D. J., 110
HIGGINS, G. M., and D. J. INGLE. Functional homeoplastic grafts of the adrenal gland of newborn rats, 380
HIRSCHMANN, H., and O. WINTERSTEINER. Estrogenic diols from urine of pregnant mares, 116
HISLOP, W. A. Treatment of marasmus by injection of extract of adrenal cortex, 675
HITCHCOCK, F. A., R. C. GRUBBS and F. A. HARTMAN. Adrenal cortical extract and oxygen consumption, 108
HOAGLAND, R. J. Addison's disease, 109
HODGSON, C. H. See Rynearson, E. H., 808
HOEHN, W. H. See Mason, H. L., 381
HOLST, J. E. Three cases of chromaffin cell tumors of the suprarenal gland, 380
Homeografts of the adrenal gland, 110
HOPKINS, H. U. See Rose, E., 392
Hormones, factors influencing effectiveness of, 679
HOUSSAY, B. A., and J. C. FASCIOLO. Humoral mechanism in hypertension, 111
HOUSSAY, B. A., and V. G. FOGLIA. Pancreatic function in phloridzin diabetes, 118
HUG, E. Adrenal secretion and potassium, 109
HUNT, H. F. See Patterson, W. B., 812
Hydatidiform mole and chorionepithelioma, hormonal findings, 531
Hyperinsulinism and cerebral changes, 388
Hyperparathyroidism, 812
Hypertension, humoral mechanism in, 111
Hyperthyroidism, 390, 391, 392
iodine, tolerance test, 120
in treatment of, 811
and pulmonary tuberculosis, 392
size and shape of heart in, 812
treatment of, with a high fat diet, 392
and vitamin B_1 tissue content, 391
and vitamin C, 391
Hypoglycemia, 810
of new-born and islands of Langerhans, 388
Hypogonadism and cryptorchidism, endocrine study of, 680
Hypoparathyroidism, parathyroid grafts in, 119
Hypophyseal, hormones, discharge of by arteries, 261
implants in salamanders, 261
transplants in pubertal emaciation, 807
Hypophysectomized, dog, blood serum amylase in, 259
rats, calorigenic action of amino acids in, 259
nitrogen and metabolism relationships in, 805
thyroxin and carbohydrate metabolism, 808
work performance and cortin, 806
Hypophysectomy, and androgenic sterols, 115
of dogs, 259
Hypophysis, anterior lobe, extract, and blood fats, 113
diabetogenic action of, 809
and glycogen of fasting rabbits, 807
ketonemia, 113
ovotrophic and folliculophasic hormones, 680
recent knowledge of, 808
antisera to preparations of prolactin, 809
antuitrin-S cutaneous test for pregnancy, 116
of armadillo, 808
assay of posterior lobe of, 384
and bile, 807
and carbohydrate metabolism, 260, 384
cellular structure of, in pigeons, 385
effect of, anterior lobe hormones of, on immature mice, 384
antithyrotropic serum on thyrotropic hormone of human, 384
incubation on oxytocic and pressor activity of, 385
electrophoresis of preparations of, 112
endoneural secretion of, 260
histophysiology of anterior lobe, 259, 260
hormones of, and ovarian hormones, 807
in the treatment of cryptorchidism, 119
and human pigmentation, 112
insufficiency and plasma proteins, 806
irradiation of, in treatment of the menopause, 112
and lactation, 805
lactogen content and vitamin deficient rations, 808
and liver fat, 806
Laurence-Moon-Biedl syndrome, 677
and ovulation, 252
and parturition, 806
pigeons as test animals for prolactin, 261
pitocin in labor, 809
posterior lobe, and intestinal segments, 807
press-juice of, 806
pressor principle, 260
and water uptake of frogs, 250
post-partum necrosis of anterior lobe, 261
pro-gonadotropic sera, 808
and renal function, 110
renal rickets and dwarfism, 259
roentgen irradiation of, 112
sexual stimulating function of, and the thyroid, 113
Simmonds's disease, 805
thyreostimulin, 390
thyroid, and folliculin, 259
and iodine, 260
in stimulation of tissue metabolism after estrogen injection, 252
thyrotropic hormone, 119
thymus and ovary, 807
and tissue metabolism, 392

and ultimobranchial body, 805
and water metabolism, 386
Hypopituitarism, diagnosis of, 386
Hypothyroidism and eclampsia, 812

INFECTIONS and the thyroid, 120
INGLE, D. J. See also Higgins, G. M., 380
INGLE, D. J. Work-performance of hypophysectomized rats treated with cortin, 806
INGLE, D. J., G. M. HIGGINS and H. W. NILSON. Homeografts of the adrenal gland in rats of inbred strains, 110
INGLE, D. J., and E. C. KENDALL. Weights of adrenal glands in rats fed different amounts of sodium and potassium, 381
INGRAM, W. R., and C. A. WINTER. Adrenalectomy, water exchange and diabetes insipidus, 251
Insulin, action of, 810
action of in cell-free extracts, 810
diabetes refractory to, and protamine zinc insulin, 388
effects of zinc and iron salts, 117
and glucose, effect of and cutting of the vagi, 387
and glycogen reserves, 262
hypoglycemia and the diabetic heart, 118
lack of response to and rôle of epinephrine in diphtheria intoxication, 674
and metrazol shock, 386
and oxidations in isolated muscle tissue 810
protamine, in the treatment of juvenile diabetes, 118
resistance and protamine zinc insulin, 387
tannate, 262
treatment of schizophrenia, 262, 388
and uric acid in the blood, 388
Intestine, small, antianemic function of, 678
Iodine, blood level in exophthalmic goiter, 812
determination of, 812
IRVING, G. W., JR. See also Du Vigneaud, V., 112.
IRVING, G. W., and V. DU VIGNEAUD. Press-juice of posterior pituitary, 806
ISBRUCH, F. Pregnancy, hydatid mole, chorionepithelioma and Aschheim-Zondek reaction, 114
ISRAEL, S. L. See Mazer, C., 677

JACOBI, M. See Rascoff, H., 388
JACOBSEN, E. Male hormones and seminal vesicles, 256
JACOBSEN, E., and J. T. CHRISTENSEN. Male hormones and seminal vesicles, 256
JAUDON, J. C. See Hartman, A. F., 810
JEMSON, J. See Allott, E. N., 389
JENKINS, C. E., and M. L. THOMSON. The distribution of iron in blood, 382
JONES, J. H. Food intake and parathyroid tetany, 811
JOSLIN, E. P. Mortality of diabetic children, 118
JOURDAN, F. See Hermann, H., 380

KARNOFSKY, D. See Addis, T., 390
KARASEK, F., and O. POUPA. Augmentation by testosterone of effect of adrenin on arteries, 533; modification of the vascular effects of adrenin by sex hormones of the opposite sex, 381
KELLY, H. T. Dermatophytosis in diabetes, 387
KENDALL, E. C. See Ingle, D. J., 381, and Mason, H. L., 381
Ketogenic activity of endocrine extracts, 806
Ketolytic activity and adrenalectomy, 251
Ketonemia produced by anterior pituitary extract, 113
KEYS, A. Response of plasma potassium level to epinephrin, 110
KILIAN, V. Estrogens in therapeutic baths, 117
KING, F. H. See Sohval, A. R., 392
KING, J. L. Pregnancy urine extract and gestation, 256
KING, J. T. Pharyngeal infections in exophthalmic goiter, 391
KITCHELL, J. R. Syphilitic diabetes, 387
KLINE, R. See Britton, S. W., 250
KOCHAKIAN, C. D., P. L. MACLACHLAN and H. D. MCEWEN. Androgens and blood lipids, 116
KOFF, A. K. See Davis, M. E., 679
KON, S. K. See Folley, S. J., 680
KREBS, H. A., and L. V. EGGLESTON. Insulin and oxidation in isolated muscle tissue, 810
KROHN, P. L., and S. ZUCKERMAN. Water metabolism and the menstrual cycle, 117
KYLLIN, E. Treatment of pubertal emaciation, 807

LACASSAGNE, A. Lymphosarcoma and estrogenic substances, 533
LACHMAN, S. E. See Greulich, W. W., 245
Lactation, and adrenal cortical hormone, 251
and anterior pituitary, 805
effect of sex hormones on, 680
and estrogens, 114, 115
lactogenic hormone in urine during, 533
LAHEY, F. H. See Perkins, H. J., 120, 812
LAIDLAW, G. F. Nesidioblastoma, islet tumor of the pancreas, 387
LAIRD, J. Lactogenic hormone in urine of lactating women, 533
LAMBIE, C. G., and V. M. TRIKOJUS. Preparation of a thyrotropic hormone, 119
LAQUEUR, E. See Dingemanse, E., 680
LARSON, E. Pressor principle of posterior pituitary, 260
effects of post pituitary on isolated intestinal segments, 807
LASZT, L., and F. VERZÁR. The B vitamins and the adrenals, 109
Laurence-Moon-Biedl syndrome, 677
LAURO, A. Hypophysis in deviation of the bile, 807
LAUSON, H. See Heller, C. G., 256
LAWRENCE, C. H., and A. M. HARRISON. Treatment of male sexual underdevelopment, 115
LAWRENCE, J. H., W. O. NELSON and H. WILSON. Roentgen irradiation of the hypophysis, 112
LEE, M. See Chown, B., 259
LEHMANN, H. Insulin in cell-free extracts, 810
LENKE, S. E. See Loewe, L., 533
LERMAN, J. Thyrotoxicosis, 119
LEW, W. See Addis, T., 390
LEWIS, J. T., and F. N. GALLO. Eserin and adrenal secretion, 109
LEWIS, L. A. See C. G. Toby, 676
LEWIS, R. A. Hyperthyroidism and vitamin C, 391
LIECHTI, H. Muscle changes in Basedow's disease, 391
LINDEMANN, W. Milk secretion and follicular hormone, 115
Lipoids and sex hormones, 252
LISSER, H. See Escamilla, R. F., 805
Liver, denervation of, and carbohydrate metabolism, 112
fat, and endocrines, 806
and pancreas, 262
function and choline, 807
glycogen and anterior pituitary, 807
LJVRAGA, P. Denervation of the liver and carbohydrate metabolism, 112
Lobelin, hyperglycemic action of, and adrenals, 531
LOEWE, L., and S. E. LENKE. Entrogenic hormone in peripheral gangrene, 533
LOEWE, S. Influence of autonomic drugs on ejaculation, 533
LOGARAS, G., and J. C. DRUMMOND. Vitamin A and the thyroid, 812
LONGUET, A. See Oumansky, V., 382
LOONEY, J. M. See Randall, L. O., 262
LUCK, J. M. See Evans, H. M., 259
LUKENS, F. D. W. See also Dohan, F. C., 809
LUKENS, F. D. W. Pancreatectomy in the goat, 810
LUN, F. Insulin tannate, 262
Lymphosarcomas and estrogenic substances, 533

MCCAHEY, J. F. Gonadotropic hormone therapy in affections of the male genitals, 117
MCCULLAGH, D. R. See Cutuly, E., 115
MCCUNE, D. J., and H. BRUCH. Osteodystrophy fibrosa, 678
MCEWEN, H. D. See Kochakian, C. D., 116
MCHENRY, E. W. See Watson, M. C., 117
MACKAY, E. M., and R. H. BARNES. Adrenalectomy and ketolytic activity, 251
MACLACHLAN, P. L. See Kochakian, C. D., 116
MCLEAN, D. L., J. H. RIDOUT and C. H. BEST. Choline and liver function, 807
MAGOUN, H. W. See Fisher, C., 806
MALAMUD, N., and L. C. GROSH. Hyperinsulinism and cerebral changes, 388
MAN, E. B. See Bogdanovitch, S. B., 252
MANFREDI, F. J. See Rojas, P., 119
MARAÑON, G. Infections and the thyroid, 120

MARAÑÓN, G., C. RICHET, M. SOURDEL and H. NETTER. Hypophysary pathogeny of disturbances of pigmentation, 112
Marasmus, treatment of, by extract of adrenal cortex, 675
Mare serum hormone, bioassay of, 253
MARGITAY-BECHT, A. Serum sodium content in myxedema, 120
MARKS, H. P., and F. G. Anterior pituitary and liver glycogen, 807
MARRIAN, G. F. See Butler, G. C., 380
MARTINS, T. Rapid test for male hormone, 533
MASON, H. L. Chemical studies of the suprarenal cortex, 381
MASON, H. L., W. H. HOEHN and E. C. KENDALL. Chemical studies of the suprarenal cortex. Structures of compounds C, D, E, F, and G, 381
MASON, L. W. Sex hormone factors in abortion and sterility, 534
MASON, V. R. See Courville, C., 805
Mastopathies, treatment with testosterone, 532
MAZER, C., S. L. ISRAEL and C. W. CHARNY. Endocrine factors in human sterility, 677
MELGAR, R., and J. L. PELUFFO. Therapy with blood serum from testicular vein in insane, 534
Mental deficiency and adiposity, 677
Menopausal disturbances, treatment with testosterone, 532
Menopause, treatment by irradiation of the pituitary, 112
Menstrual, cycle and blood regeneration, 382
and red blood cell count, 116
and water metabolism, 117
disturbances, and hormones, 257
treatment with testosterone, 532
Menstruation, orally induced, 679
tissue loss from endometrium, 117
Merthiolate, action of, and gonadotropic effects, 258
MESSINGER, W. J., M. H. PRESBERG and M. D. FELLOWS. Pregnancy test of Visscher and Bowman, 117
Metabolism, basal, and adrenal cortical extract, 108
nervous and hormonal regulation of, 382
body temperature, adrenals and exposure to cold, 675
calcium and phosphorus in diseases of thyroparathyroid apparatus, 811
carbohydrate, and denervation of the liver, 112
and the hypophysis, 260
creatine-creatinine, and purines, 676
fat, and estrone, 258
and hypophysectomy, 259, 805
of liver and kidney, effect of amniotin or theelin, 252
tissue, in normal, spayed, spayed-thyroidectomized and hypophysectomized female rats, 392
thyroid, hypophysis and estrogens, 252
water, and the menstrual cycle, 117
Metrazol and insulin shock, 386
Metrorrhagias, 252
MIMPRISS, T. W. Treatment of retention of the testis, 534
MINTZ, M. See Geist, S. H., 680
MIRSKY, I. A. See Soskin, S., 392
MISSIURO, V., D. B. DILL and H. T. EDWARDS. Adrenal cortical extract, 111
Mongolism, advanced paternal age in cause of, 676
MOORE, R. A. Congenital aplasia of the islands of Langerhans with diabetes mellitus, 262
MORIN, G. See Hermann, H., 380
Morphine poisoning and thyroid, 120
MOSINGER, M. See Roussy, G. W., 260
MÜLLER, C. Hypophysis, thymus and ovary, 807
hypophyseal and ovarian hormones, 807
MÜLLER, O. H. See Hall, V. E., 251
MUÑOZ, J. M. Anterior pituitary extract and blood fats, 113
Muscular development and androgens, 256
work and epinephrine, 531
MUTOW, T. Influence of gestation-lactation and some pathological conditions of the weight of the suprarenal glands in the albino rat, 381
Myxedema, serum sodium content in, 120

NASSET, E. S. Enterocrinin, 112
NELSON, F. W. See Cartland, G. F., 253
NELSON, W. O. See Lawrence, J. H., 112
Nesidioblastoma, 387
NETTER, H. See Marañón, G., 112
NICODEMUS, R. E. See Patterson, W. B., 812
Nicotine, blood pressure and adrenalectomy, 676
epinephrine and adrenals, 676
NILSON, H. W. See Ingle, D. J., 110
Nor-epinephrine as a mediator in the sympathetic system, 111
NORRIS, E. H. Arrhenoblastoma, 110
NORTHWAY, F. J. See Dunn, R. D., 115

OBESITY, endocrine, in children, 677
Obstetrical complications, progestin in, 255
OLDHAM, F. K. Hypophysis of the armadillo, 808
Oophorectomy and the climacteric, 257
Osteitis-fibrosa and parathyroid tumor, 389
Osteodystrophy fibrosa, 678
Ostrea virginica, physiology of reproduction of, 532
OUMANSKY, V., and A. LONGUET. An endocrine cardiac syndrome, 382
Ovarian, hormones, 254
tumor, malignant, and endocrinological effects, 110
pregnancy complicated by, 383
and folliculin, 253
Ovaries, occurrence and action of adrenalin in, 114
Ovary, thymus and hypophysis, 807
OVERHOLSER, M. D. See Barks, O. L., 382
Ovotrophic hormones, 680
Ovulation, electrical concomitant of, 257
experimental production of, 679
follicular development and gonadotropins, 255
and hypophysis, 252
Oxygen consumption and adrenal cortical extract, 108

PAGE, E. W. The effect of eclamptic blood upon the urinary output and blood pressure of human recipients, 384
Pancreas, chromaffin and argentaffine cells, 262
dextrose injection and the islands of Langerhans, 388
and fat deposition in liver, 262
function of, and phloridzin diabetes, 118
hypertrophy and hyperplasia of the islands of Langerhans and hypoglycemia of newborn, 388
islet tumor of, 387
origin of the islands of Langerhans, 389
Pancreatectomy in the goat, 810
PAPANICOLAOU, G. N., and E. A. FALK. Androgens and muscular development, 256
PAPENHEIM, E. See Schur, H. Action of insulin, 810
Parathyroid extracts and variations in bone growth, 389
grafts in hypoparathyroidism, 119
hormone, calcium gluconate and thrombocytopenic purpura hemorrhagica, 389
hyperfunction, 810, 812
tetany, 118
and food intake, 811
thyroparathyroid apparatus, diseases of, 811
tumor and generalized osteitis-fibrosa, 389
PARKES, A. S. See Deanesly, 679
PATTERSON, W. B., R. E. NICODEMUS and H. F. HUNT. Hypothyroidism and eclampsia, 812
PATTISON, A. R. D., and W. G. A. SWAN. Surgical treatment of pituitary basophilism, 385
PEDERSEN-BJERGAARD, K. See Anersen, I., 678
PELUFFO, J. L. See Melgar, R., 534
PENCHARZ, R. I. See Evans, H. M., 259
PERALTA, R. A., and E. COLOMBO. Folliculin during pregnancy, 257
PERKINS, H. J., and F. H. LAHEY. Iodine tolerance test in diagnosis of hyperthyroidism, 120
exophthalmic goiter and eclampsia, 812
PHELPS, D. See Diaz, J. T., 109, 254
PHILLIPS, R. A. See Farr, L. E., 259
Pigmentation, human, and the hypophysis, 112
PINKSTON, J. O. See Greer, C. M., 111
PIZZOLATO, P. See Beard, H. H., 676
PLANT, O. H., and D. SLAUGHTER. Thyroid feeding and morphine poisoning, 120
Plasma potassium level and administration of epinephrin, 110
proteins in pituitary insufficiency, 806
POO, L. J. See Addis, T., 390
POUPA, O. See Karasek, F., 381, 533
POWELL, T. O. Hormonal tests and histologic sections in tumor of testis, 257
Pregnancy, and adrenal cortical hormone, 251

effects of folliculin during, 257
and glucemic response to gonadotropins, 260
and ovarian tumor, 383
test, antuitrin-S cutaneous, 116
Aschheim-Zondek, 114
intradermal, 806
Visscher-Bowman, 115, 117
skin, 116, 119
urine extract and gestation, 256
Prenatal development and androgen, 533
Prepuce, glands of, and male hormone, 253
PRESBERG, M. H. See Messinger, W. J., 117
Progesterone, treatment of abortion with, 254
Progestin in obstetrical complications, 255
Prolan and magnesium content of serum, 252
Prolactin, anti sera to, 808
test for, 261, 386
Prostatic hypertrophy, 679
Protein, body, thyroxine and dinitrophenol, 390
Pruitus vulvae, estrogen in treatment of, 534
Psychosis, therapy with blood serum from testicular vein, 534
Purines and creatine-creatinine metabolism, 676

RANDALL, L. O., D. E. CAMERON and J. M. LOONEY. Blood lipids in insulin treatment of schizophrenia, 262
RANSON, S. W. See Fisher, C., 806.
RASCOFF, H., J. S. BEILLY and M. JACOBI. Hypoglycemia of new-born and hypertrophy and hyperplasia of islands of Langerhans, 388
RAVINA, A. See Buillard, H., 253
RAWSON, R. W., and P. STARR. Height of thyroid epithelium, assay of thyrotropic substance, 391
REBOUL, J. See Rock, J., 257
REECE, R. P., C. W. TURNER, I. L. HATHAWAY and H. P. DAVIS. Lactagen content of pituitary with vitamin deficient diets, 808
REFORZO, M. J. Thyroid and sexual stimulating function of the hypophysis, 113
REICHERT, F. L. See Dandy, W. E., 259
REIFFERSCHEID, W. Estrogen in treatment of pruritus vulvae, 534
REILLY, W. A., and F. S. SMYTH. Cretinoid epiphyseal dysgenesis, 392
REINER, M. See Sohval, A. R., 392
Renal function, adrenalectomy, post-pituitary and cortico-adrenal extracts, 110
RICHTER, C. P. Factors determining voluntary ingestion of water in normals and in individuals with maximum diabetes insipidus, 385
RICHET, C. See Marañón, G., 112
Rickets, renal, and dwarfism as a pituitary disease, 259
RIDDLE, O. See Schooley, J. P., 385
RIDOUT, J. H. See Best, C. H., 262, and McLean, D. L., 807
RIETTI, C. T. Ketonemia produced by anterior pituitary extract, 113
RING, G. C. Metabolism, body temperature, adrenals and exposure to cold, 675
ROCK, J., J. REBOUL and H. C. WIGGERS. Electrical concomitant of ovulation, 257
ROJAS, P., and F. J. MANFREDI. Parathyroid grafts in hypoparathyroidism, 119
ROOT, H. F. Insulin hypoglycemia and the diabetic heart, 118
ROSE, E., and H. U. HOPKINS. Hyperthyroidism and pulmonary tuberculosis, 392
ROSENBERG, E. F. Effect of insulin on concentration of uric acid in the blood, 388
ROTHNEY, J. W. M. See Dearborn, W. F., 245
ROUSSY, G. W., and M. MOSINGER. Endoneural secretion of hypophysis, 260
ROWLANDS, I. W. Antigonadotropic sera, 808
RUSSELL, J. A. Hypophysectomy, anterior pituitary extracts and carbohydrate metabolism, 260
thyroxin and carbohydrate metabolism, 808
RUST, W. Menstrual disturbances and hormones, 257
RYNEARSON, E. H., and C. H. HODGSON. Anterior lobe of the hypophysis, 808

SALMON, U. J. See Geist, S. H., 680
SAMMARTINO, R. See Del Castillo, B. E., 113
SAND, K. About the hormonal treatment of cryptorchism, 383
SATAKE, Y., H. SATO and K. ABE. Epinephrine output rate in dogs after sodium nitrite injection, 381
SATOW, Y. Action of adrenalin on toad's heart, 530
influence of application of cold to the dog's body upon the blood pressure and the blood sugar, with special reference to the augmented epinephrine secretion, 381
replacement of epinephrin and the splanchnic nerves, 530
SCHAEFER, K. Hypophyseal implants in salamanders, 261
SCHMID-BURGK, L. Unilateral oophorectomy and the climacteric, 257
SCHOOLEY, J. P., and O. RIDDLE. The morphological basis of pituitary function in pigeons, 385
Schizophrenia, insulin treatment of, 262, 388
SCHUR, H., and E. PAPENHEIM. Action of insulin, 810
SEALOCK, R. R. See Du Vigneaud, V., 112
SELYE, H. Adrenalin lung edema, 530
Seminal vesicles and male hormones, 256
SEVRINGHAUS, E. L. See Heller, C. G., 256
Sex, hormones, factors in abortion and sterility, 534
effect on lactation, 680
and lipoids, 252
synthetic, and menstruation, 679
treatment of endoangiitis and ulcers with, 258
organs, female, and thyroid, 390
Sexual underdevelopment, male, treatment of, 115
SHAW, F. H. Estimation of adrenalin, 676
SHEEHAN, H. L. Post-partum necrosis of anterior pituitary, 261
SHUTTLEWORTH, F. K. See Dearborn, W. F., 245, and Greulich, W. W., 245
SIGURJONSSON, J. Determination of iodine in sheep thyroid, 812
SILVETTE, H. See also Britton, S. W., 250
SILVETTE, H., and S. W. BRITTON. Renal function, adrenalectomy, post-pituitary and cortico-adrenal extracts, 110
SIMEONE, F. A. Sensitization of the adrenal gland, 251
Simmonds's disease, 805
SIMPSON, S. L. Desoxycorticosterone acetate in Addison's disease, 676
Skeleton and thyroid, 390
SLAUGHTER, D. See Plant, O. H., 120
SMYTH, F. S. See Reilly, W. A., 392
SOHVAL, A. R., F. H. KING and M. REINER. Creatine tolerance test and diagnosis of Grave's disease, 392
SOSKIN, S., and I. A. MIRSKY. Treatment of hyperthyroidism with a high fat diet, 392
SOULE, S. D. Estrogens in maternal and fetal circulation, 117
SOURDEL, M. See Marañón, G., 112
Splanchnic nerves, adrenals and work capacity, 531
STARR, P. See Rawson, R. W., 391
Status lymphaticus, 675
STEIGER, M., and T. REICHSTEIN. Chemical structure of corticosterone, 676
Sterility, endocrine factors in, 677
sex hormone factors in, 534
treatment of, 254
of women, 114
STONER, H. C. See Evans, H. M., 259
STÖRTEBECKER, T. P. Estrone and fat metabolism, 258
STREIT, K. Activity of follicle hormone in initiating labor in women, 383
STURGIS, S. H. Duration of secretory activity in the glands of the decidua vera, 383
SWAN, W. G. A. See Pattison, A. R. D., 385
SWINTON, N. W. Post-operative parathyroid tetany, 118
Sympathin. See adrenalin
SYOZI, K. Epinephrin load, ammonium chloride and magnesium sulphate, 531
epinephrin load and muscular work, 531
work capacity, adrenals and splanchnic nerves, 531
Syphilitic diabetes, 387

TAKUJI, H. See Wada, M., 676
TAUBENHAUS, M. Protamine zinc insulin and diabetes refractory to insulin, 388
TEITGE, H. Endoangiitis obliterans, ulcers and sex hormones, 258

TE LINDE, R. W. Treatment of vaginitis with estrogen, 258
Temperature, adrenals and metabolism, 675
TEMPLETON, H. J., and S. R. TRUMAN. Endocrine therapy in acne vulgaris, 678
Testes, descent of, effect of male hormone, 532
hormone treatment for undescended, 383
treatment of retention of, 534
tumors of, 257, 258
Testicular vein, blood serum from in treatment of psychosis, 534
Tetany, post-operative parathyroid, 118
Therapeutic baths, estrogens in, 117
THOMSON, M. L. See Jenkins, C. E., 382
Thrombocytopenic purpura hemorrhagica, parathyroid hormone and calcium gluconate in treatment of, 389
Thymus, hypophysis and ovary, 807
Thyroid, epithelium, measurement of height of, 391
and female sex organs, 390
hyperthyroidism, use of iodine in treatment of, 811
hypophysis, and folliculin, 259
and iodine, 260
and tissue metabolism after estrogen injection, 252
and infections, 120
and infundibular nerve centers, 811
and metropathia hemorrhagica, 252
and morphine poisoning, 120
and pancreatic diabetes, 809
and sexual stimulating function of the hypophysis, 113
and the skeleton, 390
and tissue metabolism, 392
and vitamin A, 812
Thyroparathyroid apparatus, diseases of, 811
Thyrotoxicosis, 119
Thyrotropic hormone, 390
assay of, 391
preparation of, 119
Thyroxine, adrenals and blood pressure, 391
and carbohydrate metabolism of hyperphysectomized rats, 808
dinitrophenol and body protein, 390
TIBA, M. See also Wada, M., 676
TIBA, M. Action of nicotine upon the blood pressure and adrenals, 676
Hyperglycemic action of lobelin and the adrenals, 531
TIBERI, R. See Alder-Mönnich, J., 390
TOBY, C. G., and L. A. LEWIS. Antagonistic factor in serum and cortin, 676
TRIKOJUS, V. M. See Lambie, C. G., 119
TRUMAN, S. R. See Templeton, H. J., 678
Tuberculosis, pulmonary, and hyperthyroidism, 392
TUKEY, G. See Adams, A. E., 384
Tumor of the pancreas, 387
testis, 257, 258
TURNER, C. W. See Bergman, A. J., 384, and Reece, R. P., 808
TURNER, H. H. Anterior pituitary gonadotropic and anterior pituitary-like hormones in the treatment of cryptorchidism, 383
Pituitary hormones in the treatment of cryptorchidism, 119

UESTÜN, Z. See Clauberg, C., 679
Ulcers, treatment with sex hormones, 258
UOTILA, U. Antianemic function of the small intestine, 678
Uterus, transplantation of, 532

VAGINITIS, treatment of, with estrogen, 258
VAICHULIS, J. A. The effect on the posterior pituitary principle of incubating beef heads at 37°C for 20-24 hours, 385
VALLE, J. R. Pigeons as test animals for prolactin, 261, 386
VAN BASILE, FR. See Van Boggaert, A., 386
VAN BOGGAERT, A., and FR. VAN BASILE. Arterial hypertension and vasopressive and gonadotropic hypophyseal hormones, 386
VAN DYKE, H. B. See Chen, G., 258
VAUGHAN, W. W. See Zollinger, R., 112
VERZÁR, F. See Laszt, L., 109
VETTER, H. See Guthmann, H., 114
VIAL, J. See Hermann, H., 380
VICTOR, J. See also Andersen, D. H., 252
VICTOR, J., and D. H. ANDERSEN. Hypophysis, thyroid and estrogens and tissue metabolism, 252
tissue metabolism, 392
Vitamin, A and the thyroid, 812
B_1 tissue content and hyperthyroidism, 391
B_2, B_4, and B_6 and the adrenals, 109
C and hyperthyroidism, 391
VOLK, M. C. See Gilligan, D. R., 810
VON HAAM, E. See Graffaguino, P., 119

WADA, M., H. TAKUJI and M. TIBA. Nicotine, epinephrin and adrenals, 676
WADE, H. K., and F. M. ADAMS. Tumors of testicle, 258
WAHRSINGER, P. B. See Frank, C. W., 116
WARREN, V. C. See Hecker, A. O., 677
WASHBURN, W. W. Hyperparathyroidism, 812
Water, exchange and adrenalectomy, 251
metabolism and menstrual cycle, 117
uptake of frogs and posterior hypophysis, 250
WATSON, M. C., and E. W. MCHENRY. Tissue loss from endometrium during menstruation, 117
WERMER, P. Hypophysis and water metabolism, 386
WHITE, M. R. Pitocin in third stage of labor, 809
WHITE, P. Protamine insulin and juvenile diabetes, 118
WIGGERS, H. C. See Rock, J., 257
WILSON, H. See Lawrence, J. H., 112
WINKLER, W. Nervous and hormonal regulation of basal metabolism, 382
WINTER, C. A. See Ingram, W. R., 251
WINTERSTEIN, J. Periodic discharge of hypophyseal hormones by arteries, 261
WINTERSTEINER, O. See Hirschmann, H., 116
WOERNER, C. A. Islands of Langerhans after continuous intravenous injection of dextrose, 388
WOLF, A. See Wortis, S. B., 389
WOLFE, J. B. See Greulich, W. W., 245
WOLFE, J. M. See Hamilton, J. B., 533
WOLF-HEIDEGGER, G. Origin of the islands of Langerhans, 389
Work capacity, adrenals and splanchnic nerves, 531
WORTIS, S. B., A. WOLF and C. G. DYKE. Xanthomatosis and the syndrome of diabetic exophthalmos dystosis, 389

XANTHOMATOSIS and diabetic exophthalmos, 389

YEAKEL, E. H., and E. W. BLANCHARD. Adrenalectomy and blood phospholipids and total fatty acids, 111
YOUNG, F. G. Antisera to preparations of prolactin, 809
diabetogenic action of anterior pituitary extracts, 809

ZOLLINGER, R., and W. W. VAUGHAN. Treatment of the menopause symptom by irradiation of the pituitary, 112
ZONDEK, H. Observations on hypopituitarism, 386
ZUCKERMAN, S. See Gutkelch, A. N., 116, and Krohn, P. L., 117

THIS NUMBER, WITH INDEX, COMPLETES VOLUME 23

DEC 22 1938

MEDICAL LIBRARY

ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

VOLUME 23 DECEMBER, 1938 NUMBER 6

CONTENTS

Diabetes Mellitus Associated with Hyperthyroidism. *Daniel P. Foster and William L. Lowrie, Jr.* 681

A Method of Assaying the Potency of Anterior Pituitary Extracts which Increase Liver Fat. *James Campbell* 692

An Evaluation of the Frank Method for the Determination of Prolan (Gonadotropic Principle) in the Urine of Nonpregnant Women. *Della G. Drips and Arnold E. Osterberg* 703

Photo-Colorimetric Method for the Determination of Androsterones in Urine. *Rudolph Neustadt* 711

The Specific Metabolic Principle of the Pituitary, and Its Relation to the Melanophore Hormone. *D. K. O'Donovan and J. B. Collip* 718

Studies of the Effects of Pituitary Extracts on Carbohydrate and Fat Metabolism. *A. H. Neufeld and J. B. Collip* 735

The Specific Dynamic Action Test in Castrated and Menopausal Women. *William M. Moffat* 747

The Influence of Various Physiological Substances on the Glycogenolysis of Surviving Rat Liver. Methods; Influence of the Bile Salts. *H. P. G. Seckel* 751

The Influence of Various Physiological Substances on the Glycogenolysis of Surviving Rat Liver. The Influence of Insulin Added in Vitro. *H. P. G. Seckel* 760

Insulin Therapy in Mental Diseases. *D. V. Conwell and C. J. Kurth* 767

The Effect of Testosterone Propionate on the Genital Tract of the Immature Female Rat. *Udall J. Salmon* 779

The Use of Testosterone Propionate in Functional Bleeding. *Samuel H. Geist, Udall J. Salmon and Joseph A. Gaines.* 784

The Influence of Estrogens in Egg Yolk Upon Avian Blood Calcium. *M. Altmann and F. B. Hutt* 793

Clinical and Laboratory Notes

Protamine Insulin. Three Practical Points. *G. B. Robson and H. Gray* 800

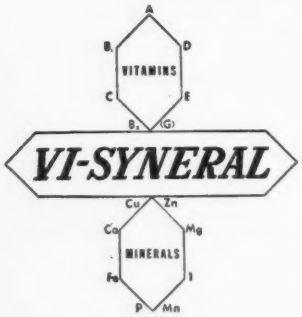
The Treatment of Acne Vulgaris with Testosterone Propionate. *Matthew Molitch* 803

Current Endocrine Literature 805

Published Monthly, Two Volumes Per Year, by The Association for the Study of Internal Secretions

Annual Subscription, $10.00 *Single Copy, $1.25*

Put the Patient's System *to Work for Him!*



The original scientific formula of Vi-Syneral, pictured above in symbolic form, was developed after 26 years of vitamin-mineral research by Dr. Casimir Funk and Dr. H. E. Dubin.

Prescribe . . .

VI-SYNERAL

Insufficient vitamin-mineral intake—that's what may be slowing up the patient's recovery! Clinical experience now shows that a great number of ailments are entirely or partially due to vitamin-mineral short rations. Without adequate amounts of these important elements, vital to the health of every cell in the body, your patient cannot make the most of your therapy. Certainly, if the average diet[1] lacks sufficient vitamins and minerals in "good health", how much greater the risk of protective food inadequacy during illness.

Put your patient's system to work for him by prescribing daily doses of Vi-Syneral*, the original multiple vitamin-mineral concentrate. Greater systemic vitality and resistance must assist recuperative power. Your specific medication acts under conditions most favorable for maximum results.

MINERALS WITH VITAMINS GIVE OPTIMAL RESULTS[2, 3]

VI-SYNERAL* contains all the definitely recognized vitamins, fortified with eight essential minerals because

. . . VITAMINS NEED ONE ANOTHER FOR BEST RESULTS
. . . MINERALS ARE ESSENTIAL TO OPTIMAL VITAMIN EFFECTIVENESS
. . . VITAMIN AND MINERAL DEFICIENCIES ARE MULTIPLE.

There is a standardized Vi-Syneral potency for every age group. Each box of 50 capsules equals the vitamin and mineral value of hundreds of pounds of fresh vegetables, fruit, milk and other foods.

* Trade Mark Reg. U.S. Pat. Off.

1. Report of League of Nations Health Comm., Dec. 6, 1935.
2. Eddy, Walter E. (Special research report on Vi-Syneral).
3. Privatera, A. T., Arch. of Ped., April, 1938.

Comprehensive Vitamin Manual and Samples Upon Request

U. S. VITAMIN CORPORATION

250 EAST 43rd STREET NEW YORK, N. Y.

NOTICE TO CONTRIBUTORS

ENDOCRINOLOGY, the official publication of The Association for the Study of Internal Secretions, comprises original articles, case reports, editorial review articles, news items, book reviews and abstracts of current literature in experimental and clinical endocrine biology and medicine.

Original articles are accepted for publication only with the understanding that they are contributed *exclusively to this journal.* Preliminary notices will not be accepted.

An abstract of less than 250 words or a copy of the summary of the article typewritten on a separate sheet with the title and author's name must accompany the manuscript. This abstract will be published in the abstract section of the journal in advance of the appearance of the complete article.

Manuscripts should be typewritten in double spacing with wide margins on one side of paper 8½ x 11 inches and should be packed flat. Tables, case histories and other subsidiary matter usually set in smaller type than the text should be typewritten on separate sheets and placed with the text in correct sequence. Footnotes and legends for figures should be typewritten on separate sheets at the end of the text copy. Footnotes should be used sparingly. Case histories and protocols should be written in clear, concise statements in the past tense and not in incomplete sentences and disjointed phrases.

Citations of literature should be numbered serially in the text. References to cited papers should not be given in the text but should be arranged in a list at the end of the paper in the order of citation and preceded by the corresponding serial number. The reference should conform to the practice of the Quarterly Cumulative Index Medicus: author's name, journal, volume (in Arabic), initial page and year. Authors are urged to verify personally the accuracy of the references, using only the original sources.

Each article should conclude with a summary of usually not more than 250 words, suited to the needs of abstract journals. This summary should be intelligible without reference to the body of the article.

A certain number of illustrations and tables will be allowed without charge. Arrangements must be made with the Editor for excess illustrations and tables.

All illustrative material should be submitted in finished form so as to admit of photographic reproduction without retouching, redrawing, labelling or the setting of marginal type.

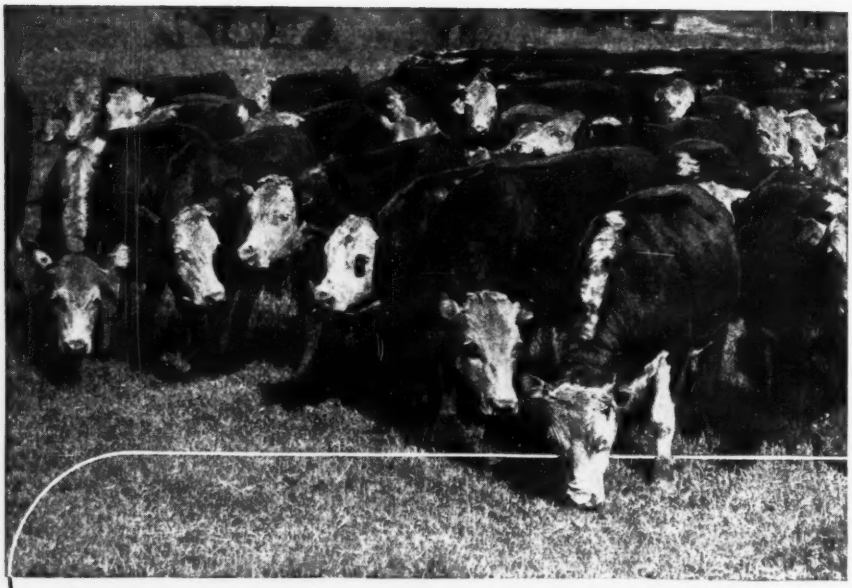
Original drawings, not photographs of drawings, should be sent. These should be made on white or blue lined heavy paper or bristol board and should be arranged to conserve vertical space.

When photographs are used for half-tone reproductions glossy prints with as sharp contrast as possible should be submitted unmounted. All unnecessary material should be trimmed away. Actual magnifications of photomicrographs should be given. In grouping illustrations it should be borne in mind that text figures after reduction cannot exceed the dimensions of the printed matter on the page, 4.66 x 7.75 inches. The preparation, arrangement and trimming of illustrations and the arrangement of tabular data so as to conserve vertical space on the page is especially urged. The author should place his full address at the top of the first page of the manuscript, and his name on the back of each photograph or drawing.

Manuscripts, correspondence relating to the editorial management and books for review should be sent by mail or by prepaid express to the Editorial Office, 25 Shattuck St., Boston, Massachusetts.

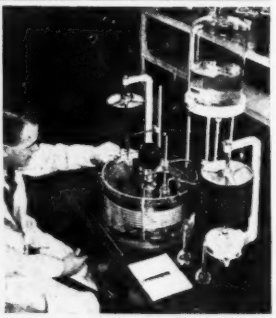
Galley proofs and engraver's proofs are sent to the author.

Reprints: A price list and order blank for reprints will be sent with the galley proofs.



This is Armour Pituitary Liquid on the Hoof

1. The Camera Cannot Show the difference in potency of these two glands. They look alike, they are equally fresh, and both are therapeutically valuable . . . yet one may be definitely more potent. That is why the standardization of Pituitary Liquid is a vitally important Armour process. The adjoining picture shows how it is done.



This Instrument measures the Muscular Contractions of a guinea pig uterus when stimulated by Armour Pituitary Liquid. Because a human uterus contracts in the same way, and because the effects of any given dosage must be accurately predetermined, this instrument is used to standardize every batch of Armour Pituitary Liquid.

The pituitary glands of these animals will become medically important in The Armour Laboratories. They will be painstakingly dissected, painstakingly transformed into Armour Pituitary Liquid (Posterior Lobe) U. S. P. by the most modern processes and techniques in use today.

Space does not permit the full story of Armour Pituitary Liquid, and its potency, standardization and dependability. Write to The Armour Laboratories, Union Stock Yards, Chicago, for complete information.

THE ARMOUR LABORATORIES

PIONEERS IN ENDOCRINOLOGY SINCE 1886

PRINTED BY GEORGE BANTA PUBLISHING COMPANY, MENASHA, WISCONSIN